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A biochemical investigation of cirrhosis.

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A BIOCHEMICAL INVESTIGATION OF CIRRHOSIS

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Introduction

Dr. Mann (1) has aptly described the liver as the "commissariat of the body". Being the largest gland in the body, the liver engages in a multiplicity of known functions and a probable host of unknown functions. Its described functions include hematopoietic processes, detoxification, reticuloendothelial functions, water balance, and the all important metabolism of food material to provide rapid utilizable energy for the tissues. The complexity and fluctuation of function must be emphasized in order to evaluate the accumulating mass of data concerning hepatic function. All the activities of this organ are not affected to the same degree by a disease process. The functional status of the liver is dependent on the physiological or pathological status of a number of other organs. Damage up to eighty percent (2) does not cause abnormal results in liver function tests performed upon experimental animals. Closely allied to the large reserve power is the remarkable regenerative power of the gland which may completely mask the pathological lesion. Diet, impaired renal function, or faulty intestinal absorption may also affect the results obtained in the various liver function tests. Hoffbauer and his co-workers (3) have recently pointed out the value of a composite study of liver functions. Several investigators (3,4,5,6,7,8) have made use of the liver biopsy in an attempt to correlate morphological change with impaired liver functions.

This paper presents a series of liver function tests made in conjunction with a micro-chemical and microscopic examination of material obtained by liver biopsy. The function tests selected were based upon the secretory and excretory functions of the liver, the detoxification process, and the roles played by carbohydrate, protein, and lipid metabolism in the diseased liver. The biopsies proved advantageous in studying the dynamics of the diseased liver when

proper precautions were used. The precautions observed were:

a - contamination of the specimens with blood was reduced to a minimum;

b - tissue for chemical analysis was placed immediately in potassium hydroxide solution and tissue weight determined;

c - specimens for pathological examination were placed in saline packs and sent to the laboratory for examination directly after the operations. Another advantage of tissue analysis lay in the fact that samples could be obtained and studied at frequent intervals during life.

The hazards of random sampling were taken into account in evaluating the results. The biopsies were always performed at the same hour of the day, on patients in a fasting state, in order to avoid the diurnal variations of liver glycogen (43). Gomori and Goldner (44) advocate great caution in judging the metabolic state of the liver on the basis of the glycogen content of biopsies. They observed variations exceeding several hundred percent in samples taken from the same lobe of a rabbit's liver. While caution should be used in judging the biopsy findings, variations of that degree are not expected in man (45); the rabbit has one of the greatest variations of all experimental animals (46). Deane and his group (47), using rats, ran a series of micro-chemical liver glycogen determinations and found the difference in readings not to exceed four percent of their mean. These investigators also showed histologically that after six to twelve hours starvation (a period similar to that of our patients), glycogen is still abundant and either distributed evenly or concentrated peripherally in the lobules. Our micro-chemical glycogen results were checked against the pathologist's report.

The data reported in this paper was obtained from sixteen patients at Louisville General Hospital; the clinical diagnosis on these patients was cirrhosis of the liver. Cirrhosis, by our definition, is a chronic disease characterized by

degeneration of the liver parenchyma with fatty destruction and necrosis of the individual cells. There is fibrous tissue proliferation and subsequent regeneration of parenchymal cells resulting in a changed lobular and vascular pattern (9, 10).

Methods

1. Total Protein:

This substance was determined on serum by a micro-Kjeldahl method employing direct nesslerization. An appropriate correction for non protein nitrogen was applied (11). The procedure was a modification of Wong (12) with peroxide replacing persulfate for the oxidation. A Universal Spectrophotometer was used to measure the density of the nesslerized solutions against an ammonium sulfate standard.

2. Albumin and Globulin:

The low temperature methanol precipitation of serum globulin as recommended by Pillemer and Hutchinson (13) was used. The results obtained by this method compare favorably with those obtained through electrophoretic analyses. Sodium sulfate precipitation of globulins (Howe's method) led to quantitative inaccuracies in determination of albumin and globulin according to Pillemer and Hutchinson (13). Our laboratory verified the reports of incomplete separation of albumin and globulin when Howe's salting out method was employed.

3. Cephalin Cholesterol Flocculation Test:

This test was performed as outlined in Kolmer and Boerner (23). The results were graded from 0 to 4 +.

4. Thymol Turbidity Test:

The procedure described by Maclagen (24) was used.

5. Colloidal Gold Test:

Gray's method (25) was employed.

6. Prothrombin:

The method of Quick as outlined in Kolmer and Boerner (27) was followed in determining prothrombin.

7. Cholesterol - free and total:

The Schoenheimer-Sperry (21) procedure employing digitonin precipitation was used.

8. Lipase:

This enzyme was determined by the method of Cherry and Crandall (26) who recommended the use of an olive oil emulsion as the substrate.

9. Bromsulphalein test:

This test was performed according to the directions stated in Kolmer and Boerner (16). When 1 cc. of dye per 10 kg. of body weight was injected intravenously, the test results were interpreted according to the following scale:

10% or less dye retention at 30 minutes - no liver impairment

20% - 40% dye retention - slight impairment

50% - 80% dye retention - moderate impairment

90% or greater dye retention - severe impairment

10. Serum Bilirubin:

The method of Malloy and Evelyn (19) was employed.

11. Hippuric Acid Excretion Test:

Quick's intravenous modification was used (17,18) in order to eliminate the factor of faulty intestinal absorption. The intravenous injection contained 1.77 gm. of sodium benzoate (equivalent to 1.5 gm. of benzoic acid) dissolved in 20 ml. of distilled water.

12. Non Protein Nitrogen:

The Haden modification of a Folin-Wu protein-free filtrate (14) was used for the determination. An aliquot of this filtrate was used to determine the nitrogen

according to the method of Koch and McMeekin (15).

13. Amylase (Diastase):

This substance was determined by the incubation of serum according to a modified Somogyi (31) procedure. The reduction power of the enzymatic degradation products of the starch was determined using the glucose method described below.

14. Glucose:

Whole blood was deproteinized with zinc sulfate and sodium hydroxide (28). The new copper reagent developed by Somogyi (29) was used to determine the reducing power of the glucose. Nelson's arsenomolybdate solution (30) was employed as the chromogenic reagent.

15. Galactose Tolerance Test:

The intravenous test was performed according to the directions of Bassett and Althausen (32) Glucose was removed from the blood samples by fermentation with yeast (33) and the filtrates were then analyzed for galactose.

16. Glycogen:

The biopsied liver tissue was analyzed for glycogen according to the method described by Wagtendonk, et al (34) with the following modifications:

a - The precipitated glycogen was placed in a vacuum dessicator overnight to insure its complete dryness;

b - The glycogen-iodine color complex was developed over a thirty minute period in a constant temperature water bath (35). Preceeding biopsy analyses the Wagtendonk method was tested with guinea pigs. Twelve liver samples from these experimental animals were analyzed for glycogen content; 1 mg. of glycogen was added to each of twelve additional samples and adequate recovery was obtained.

Since, in the Wagtendonk method, the tissue glycogen was compared against

a standard glycogen solution, the purity of that solution had to be determined. The standard contained 100 mg. of glycogen (Eastman Kodak Co.) dissolved in 100 cc. of 35% potassium hydroxide. The purity of the glycogen was determined as follows: Glycogen was precipitated from 5 cc. of the standard solution (100 mg. %) with 10-15 cc. of 95% ethanol as the precipitating agent. This reaction was continued overnight in Pyrex testtubes. Addition of a 1% aqueous solution of sodium sulfate (36) seemed unnecessary to insure complete precipitation. The tubes were centrifuged at high speed, the supernatant decanted, and the alcohol fumes expelled by heat or aspiration in a vacuum dessicator. The precipitated glycogen was subject to acid hydrolysis with 5-7.5 cc. of normal hydrochloric acid (2.2%) for a two hour period in a boiling water bath. Sahynn and Alsberg (37) have determined that hydrolysis with normal hydrochloric acid is completed in two hours. It is important to replace the liquid boiled off during hydrolysis in order to prevent charring. Sahyun (38) used tin foil to cover the mouth of the test tube while Good, Kramer, and Somogyi (39) recommended an air condenser. We found the addition of a few drops of water at thirty minute intervals prevented any signs of charring. Sjogren and his group (40) have clearly demonstrated the effect of acid concentration on the hydrolysis of glycogen as shown in the following graphs:

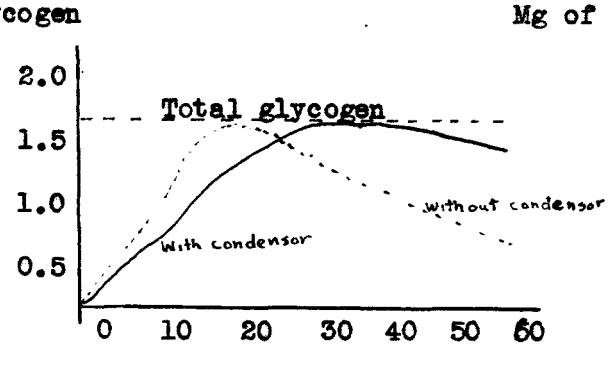


Fig. I - Hydrolysis of glycogen with 5 N sulfuric acid

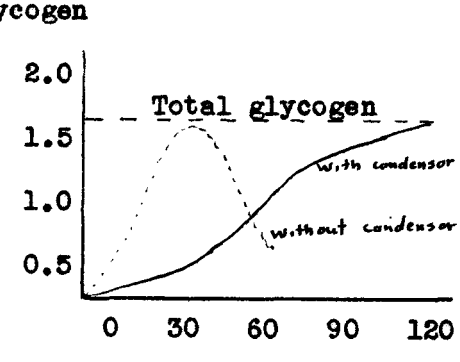


Fig. II - Hydrolysis of glycogen with 0.75 N sulfuric acid

The acid hydrolysate was transferred to a 50 ml. volumetric flask with washing and neutralized with normal sodium hydroxide using phenolphthalein as the indicator. The sample was then made up to volume with distilled water. Aliquot portions (2 ml.) of this solution were tested for glucose content according to the Somogyi method previously mentioned. The results were calculated with the following formula:

$$\left(\frac{\text{density x}}{\text{density standard}}\right)\left(\begin{array}{c}\text{mg. of glucose}\\ \text{in the}\\ \text{standard}\end{array}\right)\left(\frac{100}{\text{mg. x used}}\right)(0.927) = \text{mg.\% glycogen.}$$

The purity was ascertained to be 98.17% and this figure was rechecked eight times. We retained the theoretical factor 0.927 for the conversion of glucose values to glycogen although Nerking (41) has questioned its validity. He argues, on the basis of experimental evidence, that the factor should approach 0.95, but no definite conclusions have been reached.

The classic principles laid down by Pfluger were followed in the tissue analyses - e.g.

1 - destruction of tissue by alkali, the glycogen remaining unaffected;

2 - precipitation of the glycogen;

3 - hydrolysis of the precipitated glycogen to glucose and subsequent determination of the amount of glucose by a standard sugar technique.

According to the reports of Scandinavian investigators (42), the normal value for glycogen from excised liver tissue was found to be 6-7%. They reported values of 5.6% glycogen upon analysis of liver tissue from cirrhotic patients.

A three day period was required to complete all the various liver function tests and biochemical determinations on a single patient.

Table of normal test values

Test	Normal range
Total protein	6.5 - 8.5 gm%
Albumin	4 - 5 gm%
Globulin	1.5 - 2.5 gm%
A/G ratio	1.5 - 2.5
Cephalin cholesterol flocculation	0 - 1+
Colloidal gold	curves not exceeding an initial value of 4
Thymol turbidity	0 - 4 units
Prothrombin	80 - 100% (clotting time 12-13 seconds)
Total cholesterol	110 - 390 mg%
Free cholesterol	70 - 230 mg%
Cholesterol esters	60 - 70 %
Lipase	0.2 - 1.0 cc N/20 sodium hydroxide
Brom sulphalein dye excretion	10% or less dye retention in 30 minute specimen
Bilirubin	0.1 - 0.5 mg%
Hippuric acid synthesis	0.7 gm sodium benzoate excreted in 1 hour
Nonprotein nitrogen	20 - 40 mg%
Amylase	80 - 180 mg%
Glucose	70 - 100 mg%
Galactose tolerance test	0 - 30 mg% galactose in 75 minute specimen
Glycogen	6 - 7 %

Discussion of Results

A - Proteins:

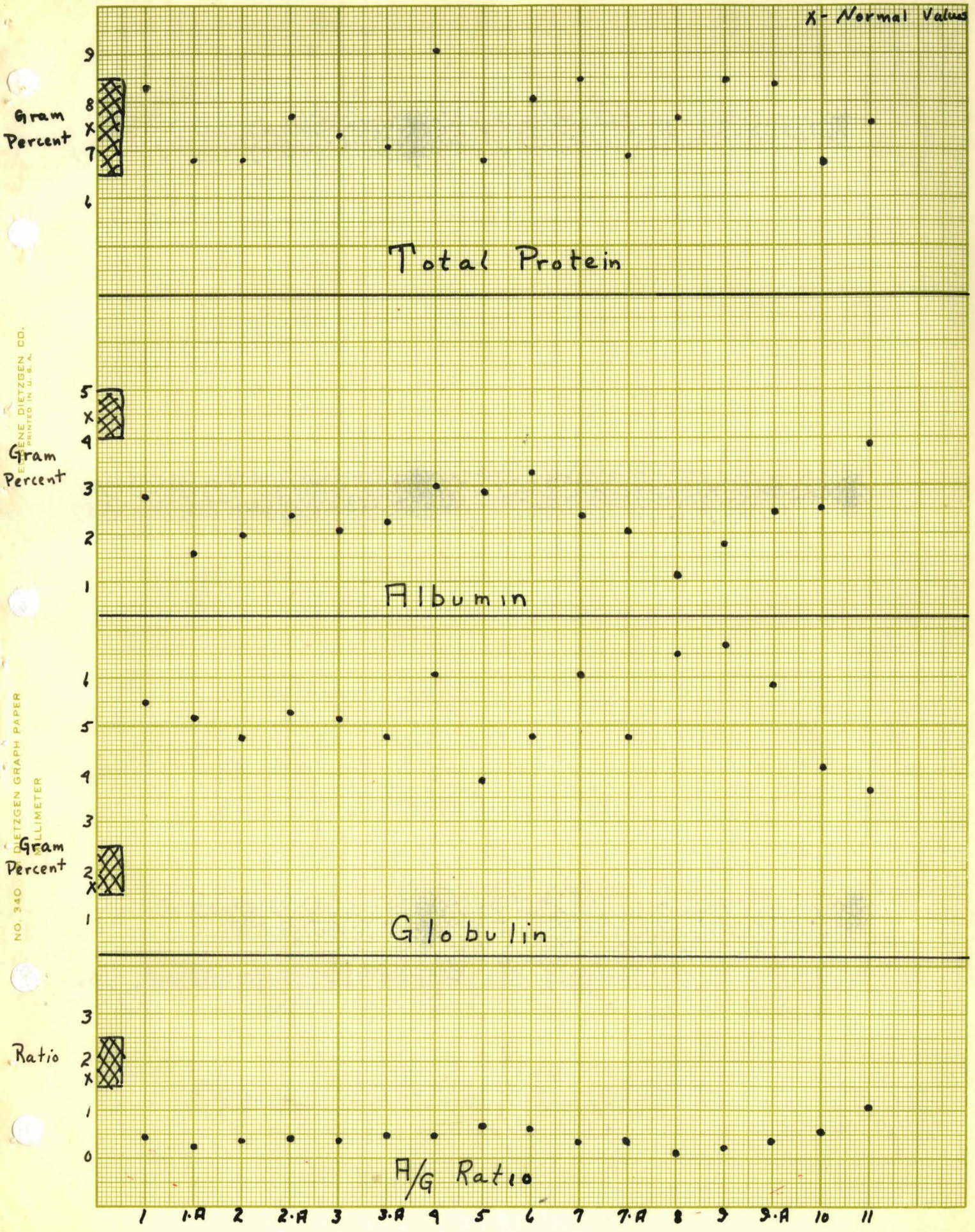
The liver is intimately concerned with the formation of the blood proteins although other tissues of the body appear to synthesize, particularly the globulin fraction. Therefore, in hepatic dys function changes in the quantity and the quality of the proteins would be expected. Electrophoretic studies (48) confirm the fact that a striking increase in the gamma globulin fraction is one of the most consistent findings in cirrhosis. In conjunction with other investigators (49, 50, 51, 52) we find a striking and consistent reversal of the A/G ratio. The decreased production of albumin (the smallest molecular weight blood protein) results in an absolute increase of gamma, beta, and alpha globulins (larger molecular weight fractions). This reversal of ratio, when considered with other test results, is valuable in diagnosing the amount of parenchymal damage (53) in a hepatic disease process. However, alteration of the ratio within itself has no specific value, since such alterations can occur in the extra-hepatic damage.

The total protein appears to stay within normal limits in cirrhosis. The slightly high value found in case 4 might be explained on the basis of the great increase in serum globulin or dehydration.

B - Specific tests for gamma globulin:

This series of tests includes cephalin cholesterol flocculation, thymol turbidity, and colloidal gold which give positive reactions in the presence of increased serum gamma globulin.

The cephalin cholesterol flocculation test was developed by Hanger in 1938 (22, 70). Electrophoretic studies (72) proved that gamma globulin isolated from normal and pathological sera showed marked cephalin flocculation activity. Hanger found that the degree of flocculation paralleled roughly the severity of the



process in cirrhosis and was negative in those instances where residual scarring was apparently the sole lesion. Our results compare favorably with those obtained by Hanger as may be seen from the following table:

Degree of flocculation	4 +	3 +	2 +	1 +	0
Hanger's cases	15	8	11	2	10
Our Cases	1	6	13	5	3

The test is simple to perform and reliable results can be obtained. The test is an index of active parenchymal damage rather than a measure of residual function (71).

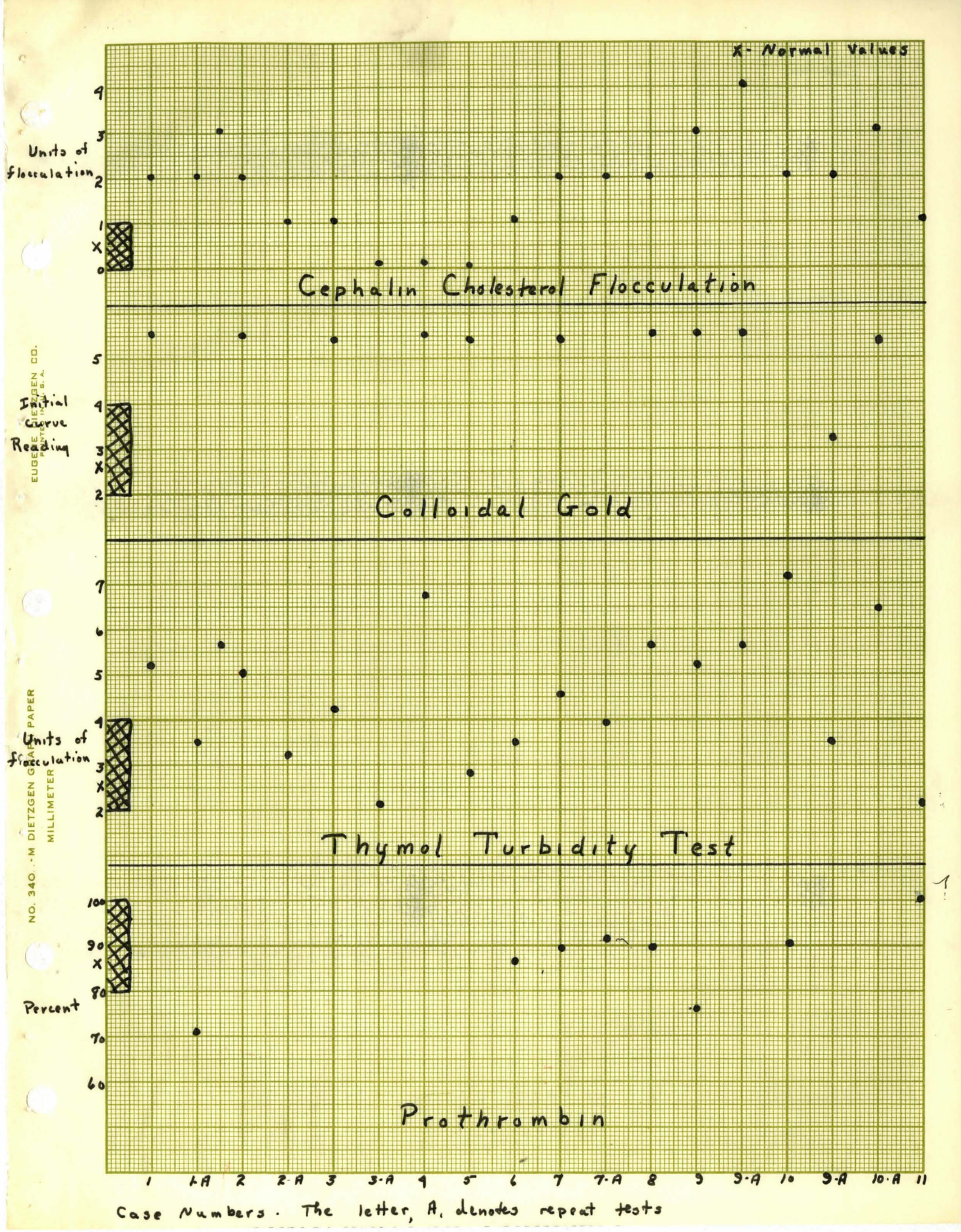
Gray (73) described the colloidal gold test in 1940. The test depends upon a qualitative change in the gamma globulin fraction and addition of electrophoretically separated albumin inhibits the reaction. In accordance with Gray we found the test remarkably sensitive yielding positive results in every case. However the technical details associated with preparation, standardization, and acidification of the gold solution render the test a difficult one to perform.

The newest of the flocculation tests is the thymol turbidity reported by Maclagen in 1944 (74). Again positive results depend upon the presence of increased gamma globulins. The test indicates acute liver damage and does not measure the degree of this involvement (75).

In several of our cases the thymol was active after the cephalin cholesterol flocculation had subsided indicating that two different activity processes were probably responsible for the positive results. Franklin and his group (76) contend that the thymol turbidity test is closely related to liver cell regeneration but our results do not confirm this idea.

C - Prothombin:

This protein is found in the globulin fraction of plasma and can be synthesized in the liver in the presence of vitamin K. Therefore impairment of liver function


X - Normal Values
Units of flocculation
9
5
2
1
X
0
Cephalin Cholesterol Flocculation
Initial Curve Reading
5
4
3
X
2
Colloidal Gold
Units of flocculation
7
6
5
4
3
X
2
Thymol Turbidity Test
Percent
100
90
X
80
70
60
Prothrombin
1
1-A
2
2-A
3
3-A
4
5
6
7
7-A
8
9
9-A
10
9-A
10-A
11
Case Numbers. The letter, A, denotes repeat tests

should result in a decrease in the prothombin level of the blood. Our results do not show this. However, the test controls are inadequate requiring a standard to be run each time on "normal" blood. We recommend that Quick's test be replaced with the test which determines prothombin time and response to vitamin K therapy. The latter test is more sensitive and reliable in our opinion.

D - Lipid Metabolism:

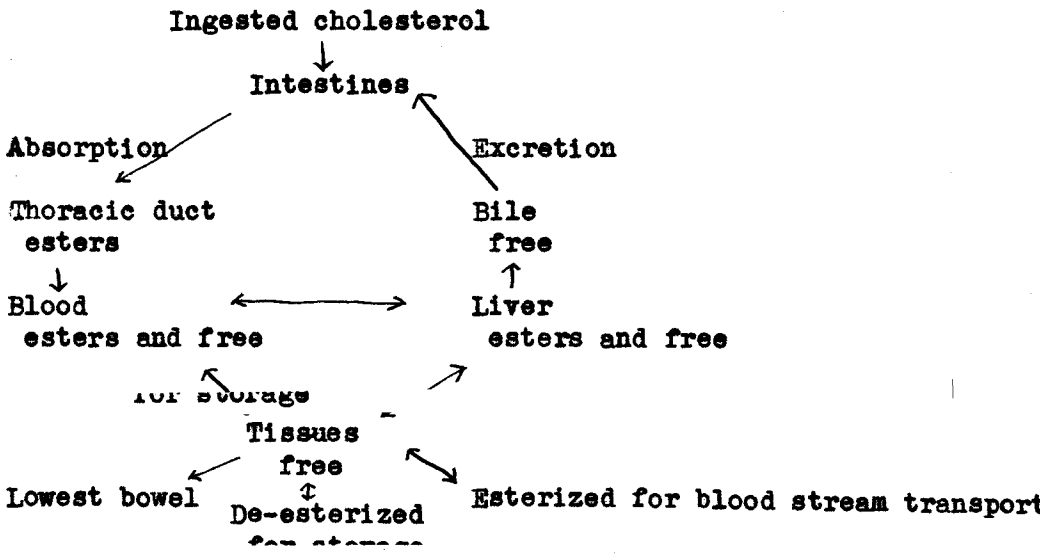
The development of more accurate methods for the determination of cholesterol have increased investigation in the relation of the liver to cholesterol metabolism. There is still a great deal of controversy on the subject but experimental evidence (65) points to the important role played by the liver in the intermediary metabolism of lipoids. This organ appears to possess the power of removing cholesterol from the blood, storing it, and probably synthesizing the compound. The liver is considered to be an active regulator of blood cholesterol and cholesterol esters. Several factors (68) influence the blood cholesterol level:

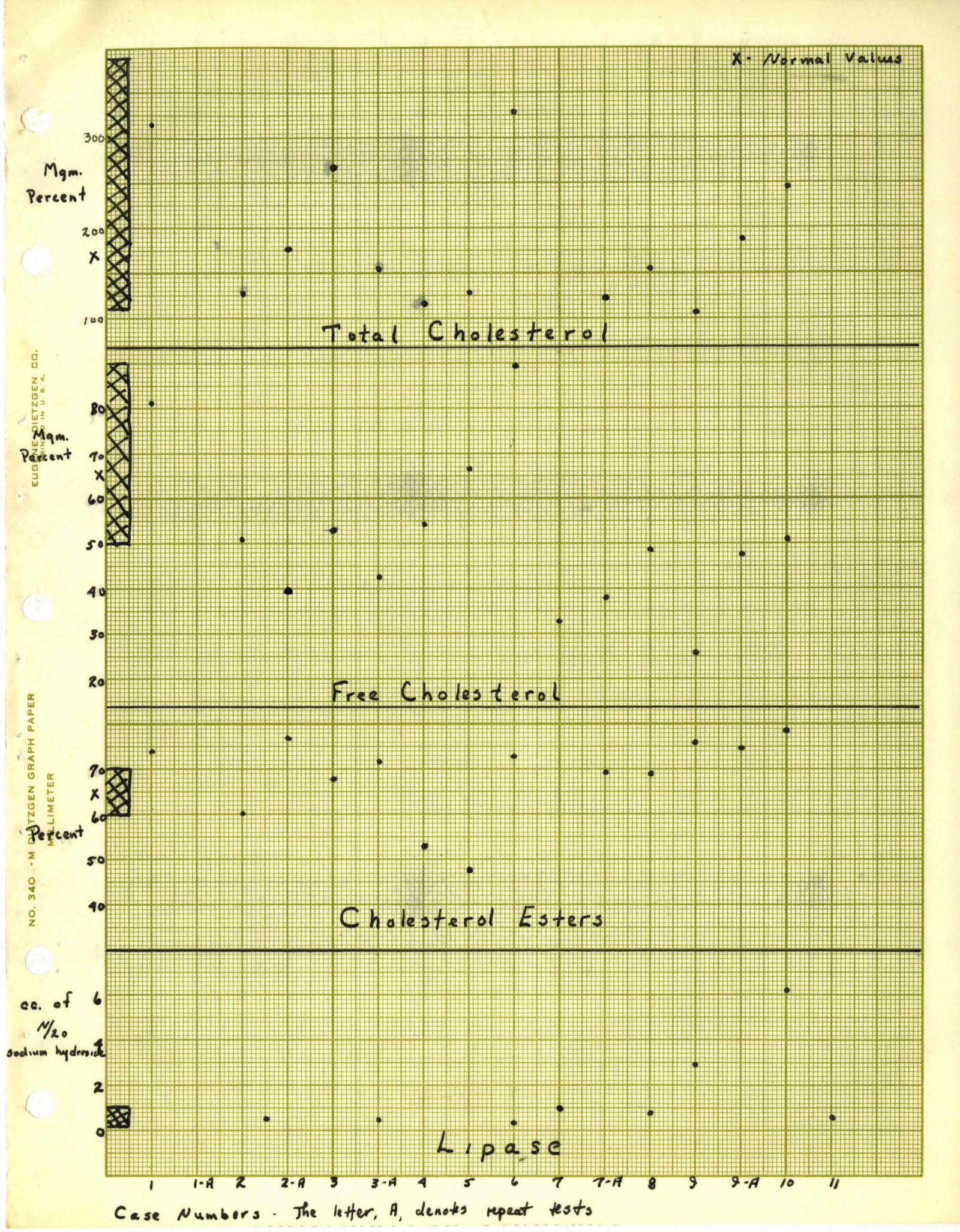
1 - diurnal variation;

2 - diet;

3 - age.

Greene and his group (66) have prepared the following chart illustrating our present knowledge of cholesterol metabolism:




X- Normal Values
Mgm. Percent
300
200
X
100
Total Cholesterol
Mgm. Percent
80
70
X
60
50
40
30
20
Free Cholesterol
Percent
70
X
60
50
40
Cholesterol Esters
cc. of N/20 sodium hydroxide
6
4
2
0
Lipase
1
1-A
2
2-A
3
3-A
4
5
6
7
7-A
8
9
9-A
10
11
Case Numbers - The letter, A, denotes repeat tests
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Many investigators (66, 67) have found that in the presence of hepatic injury the cholesterol esters of the blood are reduced in amount or entirely absent. This seems to be true in acute diseases but not in the chronic processes - cirrhosis. In our results only two cases out of sixteen show a slightly lowered blood cholesterol ester level. Epstein (69) has reported a hyper cholesterolemia, both total and ester, observed in regeneration and healing of the diseased liver. Case 6 in our series may be an example of this healing effect.

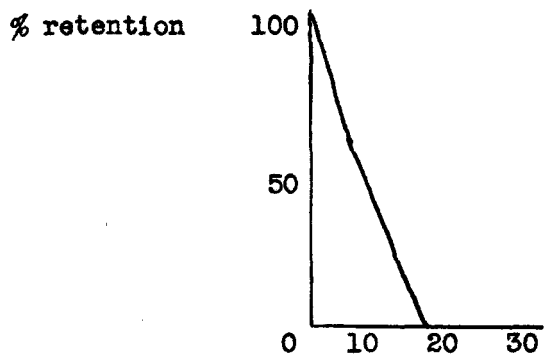
The enzyme lipase was also investigated. In 25% of our cases a rise was exhibited indicating pancreatic involvement associated with hepatic damage.

E - Excretory function tested with bromsulphalein dye:

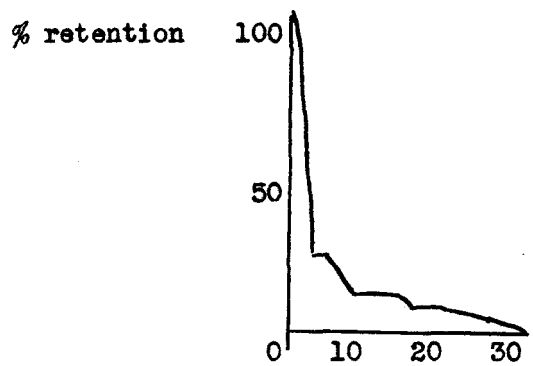
In 1924 Rosenthal (54) studied the physiological behavior of various halogenated phthalein dyes. These dyes when injected intravenously were removed by the liver and excreted in the bile. Continued research led Rosenthal and White (55) to the conclusion that the sulfonated phenoltetrahalogen phthalein group of dyes was the most satisfactory and of this group bromsulphalein possessed the best test requirements. Positive results obtained with this test indicate impaired liver function. However, Rosenthal warns that, while test results may be interpreted quantitatively from a function standpoint, the relation between function and extent of pathological lesion may be widely divergent depending on the type of lesion and its duration. This is particularly true in chronic liver disease where regeneration of cells may partially restore hepatic function, although diffuse scar tissue may be present. Our results illustrate this effect; only 12.5% of our cases demonstrated moderately severe hepatic damage by the bromsulphalein test. However, our other laboratory results indicate a much larger percentage would be expected to show decreased excretion of the dye.

Soffer (56) explains that the dye is excreted specifically by the liver,

although perhaps not quantitatively as there is some evidence that the reticulo-endothelial system is capable of taking up the dye. The test is relatively easy to perform and presents interesting evidence of liver regeneration in our group-N. B. cases 2 and 3. However Rosenthal's method has been criticized because of its lack of sensitivity (57). Macdonald (58) recommends a serial bromsulphalein test and graphically depicts the limitations of the thirty minute test based upon experimental evidence:



Normal liver function



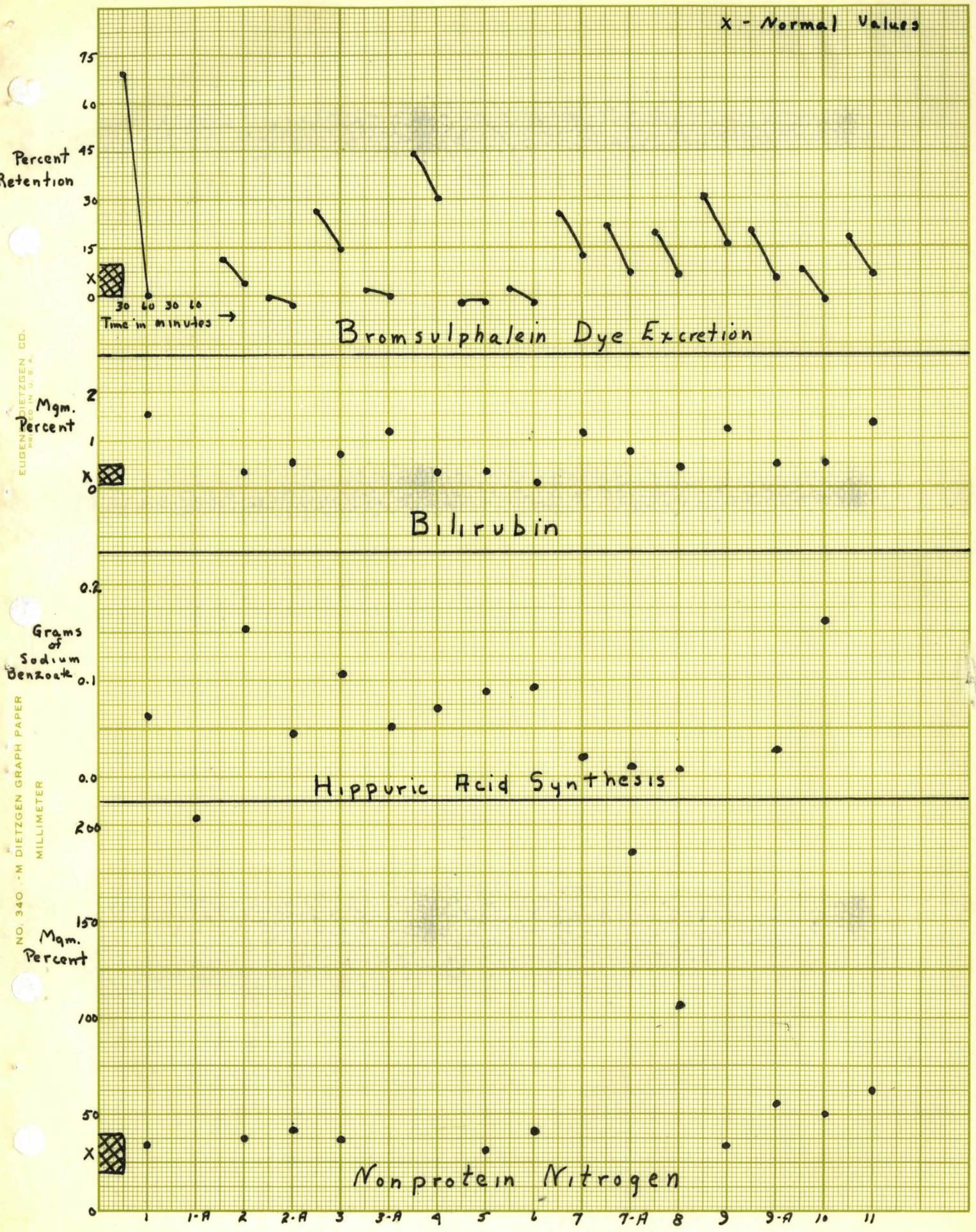
Poor liver risk, althou results within normal range

F - Secretory function of the liver:

The Kupfer cells of the liver are generally conceded to be the site of formation of bilirubin from hemoglobin. The liver epithelial cells function to excrete into the bile canaliculae the bilirubin carried by the blood stream. All of these mechanisms seem to escape severe damage in cirrhosis. Although the serum bilirubin shows slight elevation from time to time, a marked increase has not been observed in this series of cases.

G - Detoxification mechanism of the liver:

In numerous publications (59, 60) Quick has published reports dealing with the conjugation of benzoic acid and glycine to form hippuric acid. In 1938 Quick (61) reported an intravenous modification which seemed to be more sensitive in detecting liver damage. He demonstrated that only the first hour urinary excretion of hippuric


X - Normal Values
Percent Retention
75
60
45
30
15
X
0
30 60 30 60
Time in minutes →
Bromsulphalein Dye Excretion
Mgm. Percent
2
1
X
0
Bilirubin
Grams of Sodium Benzoate
0.2
0.1
0.0
Hippuric Acid Synthesis
200
150
Mgm. Percent
100
50
X
0
Nonprotein Nitrogen
1
1-A
2
2-A
3
3-A
4
5
6
7
7-A
8
9
9-A
10
11
Case number - The letter, A, denotes repeat tests

acid measures the maximum capacity of the normal liver to synthesize this substance; the second hour excretion contained much smaller amounts of hippuric acid. However in the diseased liver the reverse is true.

This test measures a metabolic as well as detoxification process occurring in the liver. The rate of hippuric acid formation is dependent primarily on the rate of glycine synthesis by the liver. White and his co-workers (62) have stated that the mechanism of hippuric acid synthesis seems to have little reserve and is easily upset. We have found this to be true and our results were similar to those reported by Hoffbauer et al (63) in a study of cirrhosis.

While the test has been sensitive and reliable in the hands of others (64), many factors influence the results. Complete cooperation on the part of the patient is required. Also conditions of dehydration (often seen in cirrhosis) and kidney involvement may retard the elimination of synthesized hippuric acid and result in low output.

G - Nonprotein nitrogen:

During the course of the disease the non protein nitrogen shows slightly increased values in our reports. A significant increase is noted in the terminal stages of the disease denoting decreased renal function.

H - Carbohydrate metabolism:

Experimental evidence indicates that galactose is metabolized specifically by the liver and that the conversion of galactose to glycogen is not dependent upon the action of insulin (77, 78, 79, 80, 81). Therefore galactose is the sugar of choice for testing the glycogenic function of the liver.

Since the liver slowly converts small amounts of the sugar to glycogen, decreased tolerance to this substance is observed in the presence of hepatic damage. In agreement with the results of Bassett and Althausen (81), our data illustrates decreased galactose tolerance in the majority of our cirrhosis patients. Marked

hyperclycemia, indicating a decreased galactose tolerance, correlates with the impaired glycogenic function, found upon chemical determination of glycogen in biopsied liver specimens in our series of cases. The galactose tolerance test appears to be a sensitive indicator of the glycogenic capacity of the liver.

Blood glucose was determined in order to observe any correlation between blood sugar level and liver glycogen. It is interesting to note that increased blood glucose values paralleled decreased glycogen biopsy results. This might indicate that the liver is still capable of synthesizing and mobilizing glycogen but unable to store it (82). It would be interesting to determine whether blood lactate increases in conjunction with the increase in glucose level and decrease in liver glycogen.

Determination of amylase provided no additional information concerning the functional state of the diseased liver.

Glycogen is generally considered to be the reserve palysaccharide of the animal world, although its presence has been demonstrated in certain bacteria and fungi. Determination of liver glycogen by chemical analyses of biopsied tissue offers valuable information concerning the effect of disease upon the liver and particularly upon the storage of this carbohydrate in the liver.

Claude Bernard first reported the existence of glycogen in 1856 and developed a method for its quantitative determination (83). Subsequent modification and improvement of the method by Pflüger and others has resulted in the present accurate and relatively simple micro-procedure.

The molecular structure of glycogen has been subjected to intensive investigation (84, 85, 86, 87, 88, 89, 90, 91, 92, 93). In the glycogen molecule the glucose residues are linked in chains by the α 1-4 glycosidic bond and the more stable α 1-6 glycosidic bond. These chains appeared to be arranged in a highly branched structure as indicated by the red-brown color that the poly-

X - Normal Values
Mgm. Percent
150
X
100
50
0
Amylase
Mgm. Percent
150
125
100
X
75
50
Glucose
Mgm. Percent
125
100
75
50
25
X
0
60 75 60 75
Time in minutes
Galactose Tolerance
X
6
5
4
3
2
1
0
Glycogen
1 1-A 2 2-A 3 3-A 4 5 6 7 7-A 8 9 9-A 10 11
Case Number - The letter, A, denotes repeat tests
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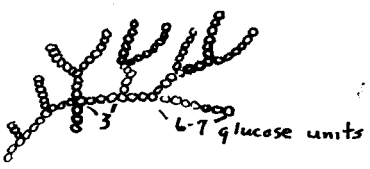
saccharide gives with iodine. Experimental evidence shows that iodine color reactions with polysaccharides are dependent upon the degree of branching and chain length of the polysaccharide. A blue color represents a relatively unbranched chain and a red color corresponds to a highly branched molecule.

Meyer has proposed the multiple branching theory to depict the ramified structure of glycogen. His theory is based upon analysis of the molecule



Structure of glycogen according to Meyer

by methylation and enzymatic degradation. Employing the Haworth and Hirst and group assay method, methylated glycogen has been found to contain one terminal glucose unit per eleven glucose residues. Glycogen is then subjected to attack by B-amylase and 47% of the molecule is degraded. The residual dextrin is again assayed and found to contain 18% terminal groups - ie, one terminal group per 5.5 glucose units. This data provides evidence for the compact structure of the residual dextrin with its multiple branching. The intact glycogen molecule contains exterior branches of 6-7 glucose units.

Since interior and exterior branches in the molecule are short, glycogen lends itself to a more compact structure which is spherical in shape as has been verified experimentally with the van't Hoff laws of osmotic pressure. The size of the native molecule is indicated by its large molecular weight which is in excess of four million.

Glycogens ingested in the diet are probably broken down completely into glucose by the amylases and glucosidases of the digestive tract. Glycogens are also degraded by α 1-4 and α 1-6 phosphorylases. In the presence of inorganic phosphate and glycogen, the phosphorylase enzymes catalyze the reaction by which

orthophosphoric acid cleaves the glycosidic linkages and yields glucose-1-phosphate. An intramolecular phosphate transfer to glucose-6-phosphate is then aided by the enzyme phosphoglucomutase.

Phosphorolysis is reversible the direction of the reaction being determined by the relative concentrations of glucose-1-phosphate and inorganic phosphate. The removal of inorganic phosphate favors glycogenesis while its addition hastens glycogenolysis. In the intact animal the removal of phosphate is accomplished by the oxidation of carbohydrate which is coupled with the regeneration of ATP and so permits the phosphate donor to be used over again in the hexokinase reaction.

The synthesis and breakdown of glycogen is affected by many factors (94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110). Only a few of these factors will be considered in this paper.

Cori has demonstrated, in vitro, that the synthesis of a polysaccharide from monosaccharides requires the presence of a small amount of the polysaccharide and that the process involves a building up of chains and branches.

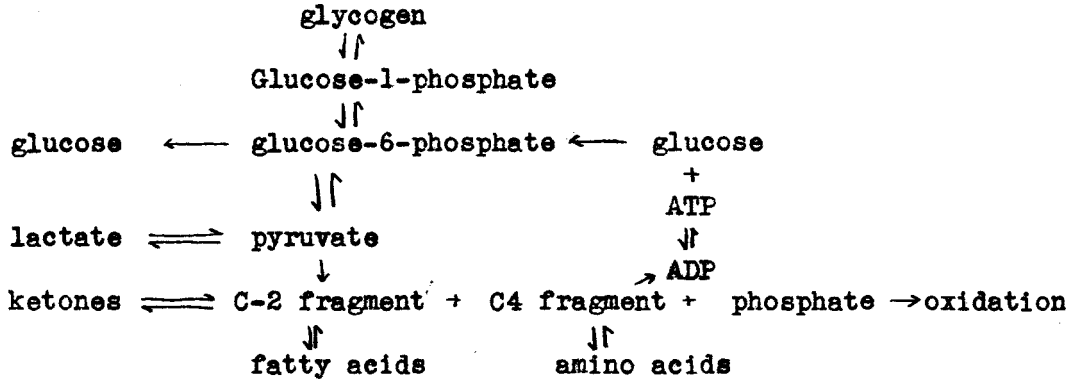
glucose-1-phosphate + terminal glucose unit (of glycogen) $\rightleftharpoons$ phosphate + α 1-4 glycosidic chain unit of polysaccharide

In addition to reaction kinetics, the hormonal balance of the body influences glycogenesis and glycogenolysis. Stetten has determined that adrenalin stimulated anaerobic glycolysis in the muscle with subsequent liberation of lactic acid which is utilized for glycogenesis in the liver. He further determined that the deposition of glycogen in the muscle is favored by insulin. The reason for this reaction can be explained on the basis of Cori's work. Cori showed that the first step in tissue utilization of glucose -

ie, glucose + ATP $\xrightarrow{\text{hexokinase}}$ glucose-6-phosphate + ADP

is inhibited by the action of an anterior pituitary extract upon the hexokinase reaction and that this inhibition is opposed by insulin.

The close integration of carbohydrate metabolism with fat and protein metabolism has been verified with isotopic studies. A simplified presentation of the inter-relation is depicted in the following diagram:



We feel that continued chemical studies of liver glycogen will contribute much valuable information concerning the function of the normal and pathological liver. Due to the various inter-relationships involved in the synthesis and storage of glycogen, we believe that greater insight into body metabolism in diseased states can be obtained. However, glycogen determinations alone can not validate any hepatic diagnoses but they must be considered in relation to other tests of liver function. Investigation of metabolic diseases in a manner similar to the procedures outlined in this paper offers untold opportunities to further the knowledge of abnormal body metabolism.

Within the limits imposed by the study of a small number of cases, we believe that liver glycogen assay affords a sensitive indication of existing hepatic pathology. However we were unable to obtain any biopsies just prior to death and therefore could not investigate this hepatic pathology and compare it with post mortem findings. Continued efforts in this field will probably furnish further insight into general body metabolism and additional data in order to secure better clinical diagnosis.

Case 1 - C.T. (m.w.) age 43

Chemical reports:

	1-9-48	3-15-48	3-17-48	4-12-48
Total protein	8.3 gm%	6.8 gm%		
Albumin	2.8gm%	1.6 gm%		
Globulin	5.5 gm%	5.2 gm%		
A/G ratio	0.5	0.3		
Cephalin cholesterol flocculation	2+	2+	2+	3+
Colloidal gold	55443211			
Thymol turbidity	5.2 units	3.5 units	3.5 units	5.6 units
Prothrombin		70.9%		
Total cholesterol	316.8 mg%			
Free cholesterol	81.6 mg%			
Cholesterol ester	74%			
Lipase	---			
Bromsulphalein dye - retention	30 min. 72.5%			
"	60 min. 3 %			
Bilirubin	1.7 mg%			
Hippuric acid	0.07 gm sodium benzoate			
Nonprotein nitrogen	37.6 mg%	214.8 mg%		
Amylase	---			
Glucose	76.4 mg%			
Galactose tolerance	---			
Glycogen	6.9%approx.	2.8%		

Pathological reports:

First biopsy (1-9-48) specimen showed considerable increase in fibrous connective tissue; remaining liver cells quite granular and swollen.

Diagnosis: cirrhosis, portal

Second biopsy (3-15-48) no pathological specimen

Death occurred 4-16-48 (hemorrhage)

Case 2 - E.B. (m.w.) age 56

Chemical reports	1-13-48	2-9-48
Total protein	6.8 gm%	7.7 gm%
Albumin	2.0 gm%	2.4 gm%
Globulin	4.8 gm%	5.3 gm%
A/G ratio	0.4	0.5
Cephalin cholesterol flocculation	2 +	1 +
Colloidal gold	553211	
Thymol turbidity	5.0 units	3.2 units
Prothrombin	---	
Total cholesterol	128.4 mg%	176.0 mg%
Free cholesterol	51.1 mg%	40.0 mg%
Cholesterol ester	60%	77%
Lipase		0.5 cc sodium hydroxide
Bromsulphalein dye - retention 30 min. " 60 min.	14.5% 5.5%	Retention 30 min. 2.5% " 60 min. negative
Bilirubin	0.5 mg%	0.7 mg%
Hippuric acid	0.16 gm sodium benzoate	0.05 gm sodium benzoate
Non protein nitrogen	41.9 mg%	44.9 mg%
Amylase	94.3 mg%	80.2 mg%
Glucose	66.5 mg%	103.3 mg%
Galactose tolerance 60 min. 75 min.	42.9 mg% 33.5 mg%	60 min. 31 mg% 75 min. 13.4 mg%
Glycogen	5.2% (approx.)	

Pathological Reports: First biopsy (1-13-48) report was cirrhosis with acute degeneration. Appearance of some of the cells suggested degeneration.

Second biopsy (2-9-48) was unsuccessful. However ascitic fluid was examined and no cancer cells were found.

Note the improvement of the liver functions dye excretion and carbohydrate metabolism in the chemical tests (2-9-48) which would suggest some cellular regeneration as reported by the pathologist (1-13-48).

Case 3 - J.S. (m.w.) Age 52

Chemical reports	1-13-48	2-9-48	2-15-
Total protein	7.3 gm%	7.1 gm%	
Albumin	2.1 gm%	2.3 gm%	
Globulin	5.2 gm%	4.8 gm%	
A/G ratio	0.4	0.5	
Cephalin cholesterol flocculation	1 +	trace	
Colloidal gold	543211		
Thymol turbidity	4.2 units	2.1 units	
Prothrombin	---		
Total cholesterol	168.4 mg%	156.0 mg%	
Free cholesterol	53.2 mg%	42.9 mg%	
Cholesterol ester	68%	72%	
Lipase		0.5 cc sodium hydroxide	
Bromsulphalein dye - 30 min.retention	28%	30 min.retention 5%	
60 min. retention	17.8%	60 min. retention 3%	
Bilirubin	0.9 mg%	1.3 mg%	
Hippuric acid	011 gm sodium benzoate	0.06 gm.sodium benzoate	
Non protein nitrogen	42.5 mg%		83 mg
Amylase	77.6 mg%	47.0 mg%	
Glucose	88.0 mg%	149.2 mg%	
Galactose tolerance 75 min.	48.6 mg%	60 min. 60.8 mg%	
		75 min. 44.9 mg%	
Glycogen	4.7% (approx.)	1.6%	

Pathological reports: First biopsy (1-13-48) showed acute degeneration of the liver. Slight amount of fibrous connective tissue present; liver cells were very granular in appearance. Second biopsy (2-9-48) report was increased fibrosis. Diagnosis: moderate cirrhosis of the liver.

An increase in liver damage can be seen in second series of chemical tests and is in accord with the interpretation of the pathologist. Death occurred 3-15-48.

Case 4 - E.G. (m.w.) Age 48

Chemical Reports:	1-20-48
Total protein	9.1 gm%
Albumin	3.0 gm%
Globulin	6.1 gm%
A/G ratio	0.5
Cephalin cholesterol flocculation	trace
Colloidal gold	5542211
Thymol turbidity	6.7 units
Prothrombin	---
Total cholesterol	116.5 mg%
Free cholesterol	54.7 mg%
Cholesterol esters	53%
Lipase	---
Bromsulphalein dye - 30 min. retention 60 min. retention	47% 33.8%
Bilirubin	0.6 mg%
Hippuric acid	0.08 gm sodium benzoate
Non protein nitrogen	---
Amylase	62.1 mg%
Glucose	104.6 mg%
Galactose tolerance 60 min.	35.4 mg%
Glycogen	5.9 % (approx.)

Pathological reports: First biopsy specimen (1-20-48) contained large amounts of fibrous connective tissue. Cytoplasm was granular and appeared to be undergoing degenerative change.

Interpretation: cirrhosis; fatty liver.

Both chemical and pathological reports indicate activity. Although the cephalin cholesterol flocculation test is negative, the thymol turbidity test indicates an active process in the diseased liver. Hippuric acid synthesis and albumin-globulin ratio results show hepatic dysfunction.

Case 5 - D.G. (m.w.) Age 7

Chemical Reports:		1-28-48
Total protein		6.8 gm%
Albumin		2.9 gm%
Globulin		3.9 gm%
A/G ratio		0.7
Cephalin Cholesterol		negative
Colloidal Gold		54321
Thymol Turbidity		2.8 units
Prothrombin		---
Total Cholesterol		128.5 mg%
Free Cholesterol		66.7 mg%
Cholesterol esters		48%
Lipase		---
Bromsulphalein dye - 30 min. retention		None
Bilirubin		0.5 mg%
Hippuric Acid		0.095 gm sodium benzoate (recovered after injection of 0.44 gm)
Nonprotein Nitrogen		35.3 mg%
Amylase		48.6 mg%
Glucose		89.4 mg%
Galactose Tolerance -	60 minutes	34.1 mg%
	75 minutes	26.5 mg%
Glycogen	first specimen	5.9%
	Second specimen	4.7%

Pathological Reports: First biopsy specimen (1-28-48) contained excessive amounts of fibrous connective tissue. Loss of normal cell architecture with swelling and granularity was reported.

Interpretation: cirrhosis (early, portal); acute degeneration (severe)

Clinical diagnosis: Detoni-Fanconi syndrome

Note degree of involvement suggested by albumin-globulin ratio and colloidal gold tests. The functions of dye excretion and hippuric acid synthesis appear normal.

Case 6 - H.B. (m.w.)

Chemical Reports:	2-18-48
Total protein	8.1 gm%
Albumin	3.3 gm%
Globulin	4.8 gm%
A/G ratio	0.7
Cephalin Cholesterol	1 +
Colloidal Gold	---
Thymol Turbidity	3.5 units
Prothrombin	85.4%
Total Cholesterol	328.0 mg%
Free Cholesterol	89.8 mg%
Cholesterol esters	73%
Lipase	0.4 cc sodium hydroxide
Bromsulphalein dye - 30 min. retention	4.0%
60 min. retention	None
Bilirubin	0.3 mg%
Hippuric Acid	0.1 gm sodium benzoate
Nonprotein Nitrogen	47 mg%
Amylase	163.7 mg%
Glucose	131.1 mg%
Galactose Tolerance - 60 minutes	63.3 mg%
75 minutes	27.8 mg%
Glycogen	2.1%

Pathological Reports: No specimen

The results of the function tests indicate a cirrhotic condition with no active lesion at the time of testing. The biopsied glycogen assay and hippuric acid synthesis are very low. Normal results obtained in the bromsulphalein test might indicate some regeneration. Negative cephalin cholesterol flocculation and thymol turbidity tests indicate no active lesion at the time of testing.

Case 7, C.R. (m.w.) Age 71

Chemical reports:	2-24-48	3-15-48
Total protein	8.5 gm%	6.9 gm%
Albumin	2.4 gm%	2.1 gm%
Globulin	6.1 gm%	4.8 gm%
A/G ratio	0.4	0.4
Cephalin Cholesterol	2 +	2 +
Colloidal Gold	543211	---
Thymol Turbidity	4.5 units	3.9 units
Prothrombin	89%	91.2%
Total Cholesterol	---	123.5 mg%
Free Cholesterol	33.2 mg%	38.2 mg%
Cholesterol esters	---	69%
Lipase	1.0 cc sodium hydroxide	
Bromsulphalein dye - 30 min. retention 60 min. retention	27.5% 15%	30 min. retention - 23% 60 min. retention - 9%
Bilirubin	1.3 mg%	0.9 mg%
Hippuric Acid	0.04 gm sodium benzoate	0.02 gm sodium benzoat
Nonprotein Nitrogen	---	189.6 mg%
Amylase	71.9 mg%	126.7 mg%
Glucose	125.9 mg%	120.8 mg%
Galactose Tolerance - 60 minutes 75 minutes	107.3 mg% 81.1 mg%	60 min. - 89.9 mg% 75 min. - 74.4 mg%
Glycogen	1.6 %	1.9%

Pathological reports: First biopsy specimen (2-24-48) showed perilobular fibrosis. Individual liver cells showed granular cytoplasm and in some areas cell borders were indistinct. Cytoplasm contained large fat vacuoles in moderate amount.

Second biopsy (3-15-48) showed all liver cells with a marked degree of swelling and granularity. There was slight diffuse fat infiltration. A moderate amount of fibrous connective tissue was reported.

Interpretation: cirrhosis, portal; fatty liver.

Case 8, O.G. (m.w.)

Chemical Reports	2-19-48	2-24-48
Total protein		7.7 gm%
Albumin		1.2 gm%
Globulin		6.5 gm%
A/G ratio		0.18
Cephalin Cholesterol	3 +	2 +
Colloidal Gold		553211
Thymol Turbidity	4.9 units	5.6 units
Prothrombin		89.4%
Total Cholesterol		155.7 mg%
Free Cholesterol		48.9 mg%
Cholesterol Esters		69%
Lipase		0.8 cc sodium hydroxide
Bromsulphalein dye	30 min. retention - 60 min. retention -	21% 10%
Bilirubin		0.6 mg%
Hippuric Acid		0.02 gm sodium benzoate
Nonprotein Nitrogen		111.3 mg%
Amylase		142.5 mg%
Glucose		---
Galactose Tolerance	60 minutes - 75 minutes -	130.9 mg% 104.8 mg%
Glycogen		---

Pathological Reports: No biopsy obtained.

Note function impairment of hippuric acid synthesis, carbohydrate metabolism and dye excretion. As the cephalin cholesterol flocculation activity decreases the thymol turbidity activity increases. The albumin is dangerously low. Death occurred 3-15-48.

Case 9, C.B. (m.w.) Age 64

Chemical Reports	2-19-48	2-26-48	3-9-48	3-15-48
Total protein		8.5 gm%		8.4 gm%
Albumin		1.8 gm%		2.5 gm%
Globulin		6.7 gm%		5.9 gm%
A/G ratio		0.27		0.4
Cephalin Cholesterol	3 +	3 +	3 +	2 +
Colloidal Gold		553211		
Thymol Turbidity	6.0 units	5.2 units	6.4 units	7.1 units
Prothrombin		76.1 %		90%
Total Cholesterol		108.7 mg%		188.2 mg%
Free Cholesterol		26.4 mg%		47.9 mg%
Cholesterol esters		76%		75%
Lipase		2.9 cc sodium hydroxide		---
Bromsulphalein dye - 30 min. retention 60 min. retention		33.5% 19.5%	30 min.retention - 22.5% 60 min.retention - 8.5%	
Bilirubin		1.4 mg%		0.7 mg%
Hippuric acid		0.1 gm sodium benzoate		0.04 gm.sodium benzoate
Nonprotein Nitrogen		4011 mg%		61.7 mg%
Amylase		181.3 mg%		116.2 mg%
Glucose		---		146.1 mg%
Galactose Tolerance - 60 minutes 75 minutes		101.2 mg% 68.2 mg%	60 min. - 75 min. -	135.4 mg% 93 mg%
Glycogen		0.5%		2.5%

Pathological Reports: First biopsy (2-26-48) section showed liver cells with considerable granulation and irregularity in size. Large groups of liver cells, believed to be lobules, were surrounded by a considerable amount of fibrous connective tissue.

Second biopsy (3-15-48) contained liver cells which showed large amount of cloudy swelling and considerable loss of detail.

Interpretation: cirrhosis, severe; acute degeneration

Note correlation between pathological and chemical reports. Second series of function tests indicate slight improvement of hepatic function.

Case 10, R.G. (m.w.) Age 44

Chemical Reports:	2-26-48	3-9-48
Total protein	6.8 gm%	
Albumin	2.6 gm%	
Globulin	4.2 gm%	
A/G Ratio	0.6	
Cephalin Cholesterol	2 +	3 +
Colloidal Gold	32211	53321
Thymol Turbidity	3.5 units	6.4 units
Prothrombin	---	
Total Cholesterol	245.2 mg%	
Free Cholesterol	51.1 mg%	
Cholesterol esters	79%	
Lipase	6.2 cc sodium hydroxide	
Bromsulphalein dye - 30 min. retention	10%	
60 min. retention	2.5%	
Bilirubin		
Hippuric acid	0.2 gm sodium benzoate	
Nonprotein Nitrogen	56.7 mg%	
Amylase	149.1 mg%	
Glucose	160.3 mg%	
Galactose Tolerance - 60 minutes	127.1 mg%	
75 minutes	43.4 mg%	
Glycogen	---	

Pathological Reports: First biopsy (2-26-48) section showed a considerable amount of granularity and irregularity in size of liver cells. Nuclei were quite large suggesting regeneration. Densely scattered fat cells apparently caused considerable atrophy of the liver.

Interpretation: fatty liver.

Clinical diagnosis: cirrhosis.

Chemical reports indicate severe involvement of the liver with decreased function in protein and carbohydrate metabolism and hippuric acid synthesis. The flocculation tests (3-9-48) indicate increased activity of the hepatic lesion.

Case 11, G.B. (m.w.)

Chemical reports:	4-21-48
Total protein	7.6 gm%
Albumin	3.9 gm%
Globulin	3.7 gm%
A/G ratio	1.1
Cephalin Cholesterol	1 +
Colloidal Gold	---
Thymol Turbidity	2.1 units
Prothrombin	100%
Total Cholesterol	---
Free Cholesterol	---
Cholesterol esters	---
Lipase	0.6 cc sodium hydroxide
Bromsulphalein dye - 30 min. retention 60 min. retention	20% 10%
Bilirubin	1.5 mg%
Hippuric Acid	0.05 gm sodium benzoate
Nonprotein Nitrogen	68.4 mg%
Amylase	81.3 mg%
Glucose	78.1 mg%
Galactose Tolerance - 60 minutes 75 minutes	5.6 mg% 2.9 mg%
Glycogen	5.5%

Summary and Conclusions

1. The glycogen assay in conjunction with the albumin-globulin ratio and galactose tolerance were found to be the most sensitive chemical tests indicative of liver pathology in this series of cases.
2. Pathological reports of cellular abnormality corresponded in a large number of cases with low glycogen values obtained from biopsies.
3. The colloidal gold test was positive in all cases.
4. Cephalin cholesterol flocculation and thymol turbidity tests showed episodes of spasmodic activity throughout the course of the disease.
5. Hippuric acid synthesis seemed to indicate a great loss of heaptic function in many of the patients but this did not correlate with the amount of damage as shown by the bromsulphalein test.
6. Total protein, amylase, prothrombin and bilirubin remained within normal range. Non protein nitrogen increased in the terminal stages of the disease probably due to loss of renal function.
7. Determination of glycogen in biopsied liver specimens provided a useful method for the continued study of the diseased organ durante vivo.

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