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# Plasma lipoprotein abnormalities in liver disease

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PLASMA LIPOPROTEIN ABNORMALITIES  
IN LIVER DISEASE

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PLASMA LIPOPROTEIN ABNORMALITIES IN LIVER DISEASE \*

by

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B.S., Virginia Polytechnic Institute, 1963

A Thesis

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## INTRODUCTION

Hepatic disease is known to alter plasma lipids and lipoproteins (1,2). In 1851, Addison and Gull described xanthelasma and xanthomata in association with liver disease (3). Shortly thereafter, Austin Flint reported "Cholesteremia" in obstructive jaundice and suggested impairment of hepatic excretory function as a possible mechanism (4). More recently, abnormal lipoprotein forms have been described in association with lipid variations in hepatic disease (2,5).

Striking increases in plasma cholesterol and phospholipids are found in biliary cirrhosis and extra-hepatic obstruction, and occasionally in acute hepatitis (1,6,7). The increase in serum cholesterol is uniquely distinguished by a gross increase in free or unesterified cholesterol (8,9), while esterified cholesterol remains normal or only slightly elevated. Increases in plasma phospholipids are proportionately greater than those in cholesterol and appear largely attributable to marked increases in lecithin (1,10,11). Triglycerides, in contrast, are normal or only slightly increased and the plasma, although icteric, is usually clear (9,12). Xanthomata and xanthelasma appear when the total lipid elevation is extreme and prolonged, and their disappearance is coincident with decreasing lipid concentrations (9).

Plasma cholesterol in portal cirrhosis and most cases of acute hepatitis is either normal or slightly reduced (6,7). Although total cholesterol is normal, an increase in the ratio of free/esterified cholesterol, similar to that in biliary obstruction, is found in the early stages of acute hepatitis (2). In patients in whom chronic liver disease develops as a result of viral hepatitis the ratio of free/esterified cholesterol returns to normal





during convalescence from the acute phase (13). Plasma phospholipids and triglycerides may be elevated during the acute phase of hepatitis with return to normal values during convalescence (2,12,14), whereas in portal cirrhosis they are characteristically normal (2,12).

Abnormal plasma lipoproteins have been described in hepatic disorders (2,5), however, their relationship to the normally circulating plasma lipoproteins is not clear. Their presence appears dependent upon the degree and extent of biliary obstruction (2). Normally, the plasma lipoproteins and particles fall into four major groups. As defined by paper electrophoresis these are the beta, alpha, and prebeta lipoproteins, and chylomicrons (15, 16). On ultracentrifugation the glyceride-rich prebeta lipoproteins and chylomicrons are found at "very low" densities ( $<1.006$ ,  $S_f >20$ ), whereas beta lipoproteins are of "low" density (1.006 to 1.063,  $S_f$  0-20) and alpha lipoproteins considered "high" density (1.063 to 1.21). Cohn fractionation (17) further distinguishes alpha lipoproteins (fraction IV) from beta lipoproteins (fraction III). This normal plasma lipoprotein pattern is altered in biliary cirrhosis, some cases of extra-hepatic obstruction, and occasionally acute hepatitis. Alpha lipoproteins are either absent or reduced on ultracentrifugal (2,18,19) or electrophoretic analyses (2,20), and only a small amount of the lipoproteins present in the beta fraction on electrophoresis (2,21), or ultracentrifugation (2,22-24) is identifiable as beta immunochemically. Associated with this apparent reduction of normal plasma lipoproteins is the appearance of abnormal lipoprotein forms. As much as 70 per cent of the plasma lipids may be found in Cohn fraction IV (normally contains alpha lipoproteins), however, these lipids are combined with protein at unusually high protein to lipid ratios and, although not precipitated by antisera to beta lipoprotein, are found to have beta mobility on electrophoresis and can be isolated with beta lipoproteins between densities



1.006 and 1.063 on preparative ultracentrifugation. In addition, most of the lipoproteins found in Cohn Fraction III (normally contains beta lipoproteins) have an increased lipid protein ratio, and though found with the beta lipoprotein fraction on electrophoresis and preparative ultracentrifugation (densities 1.006 to 1.063), are not precipitated by antisera specific for beta lipoprotein (2,5).

The nature of these abnormal plasma lipoproteins has been a matter of considerable debate. Russ, Raymunt, and Barr (5) characterized three separate abnormal beta lipoproteins on the basis of density gradient analyses, protein/lipid ratios, and Cohn fractionation, and suggested that in obstructive jaundice most of the lipids are combined atypically with beta globulins. Kunkel and Ahrens (21) studied sera from eight patients with biliary cirrhosis electrophoretically, and found increases in the beta globulin fraction to be directly related to the total lipid concentration.

DeLalla et al. (25), using antisera prepared in rabbits against human alpha lipoproteins, found evidence for the existence of alpha lipoproteins of density less than 1.063 in the plasma of a patient with biliary cirrhosis.

Furman et al. (18), suggested that the abnormal lipoproteins result from an atypical combination of plasma alpha and beta lipoproteins. When plasma samples from patients with biliary obstruction were subjected to in vitro sonic oscillation, a marked increase in the plasma lipoprotein fraction of density greater than 1.063 was observed. On the basis of this and an earlier study in which they found plasma alpha lipoprotein concentration to vary with cholesterol ester concentration, the same authors suggested that a shift in the densities of the plasma alpha lipoproteins occurred with the increasing



lipid concentrations in biliary obstruction.

Recently, Lemaire et al. (26,27), found that the phospholipids in sera from patients with obstructive jaundice are trichlorethylene extractable, whereas in subjects with either non-icteric sera or hepatocellular disease, the phospholipids are not trichlorethylene extractable. In those patients with obstructive jaundice, plasma alpha lipoproteins were absent on paper electrophoresis, however, the abnormal readily extractable lipoproteins were found to have an antigenic specificity "close to that" of plasma alpha lipoproteins.

The normal plasma lipoprotein pattern may also be altered by hepatocellular disease, here however, no abnormal lipoprotein forms have been described (2). Pierce and Gofman (24) reported elevations in the  $S_f$  10-20 lipoprotein class in portal cirrhosis, and elevations in the  $S_f$  0-100 lipoprotein class in acute hepatitis. Plasma alpha lipoprotein concentrations were reduced on Cohn fractionation in patients with acute hepatitis (2).

Recently, Fredrickson and Levy have demonstrated the value of immunoelectrophoretic methods in the identification and classification of plasma lipoproteins (28). These techniques permit characterization of the lipoproteins based on their highly specific peptide moieties. The study of lipoprotein alterations in liver disease has been complicated by disturbances in lipid metabolism which could easily alter the physical and chemical properties of the lipoproteins without changing their antigenicity, a function of the peptide moiety. Current understanding of the nature and function of the plasma lipoproteins requires that classification of a lipoprotein as either normal or abnormal must in part be based on proper antigenic classification of its peptide moiety.



It is the purpose of the present study to combine the techniques of ultracentrifugation and electrophoresis with those of immuno-electrophoresis and immunochemical precipitation in order to:

1. Better characterize the alterations in plasma lipoproteins associated with impairment of hepatic histology and function.
2. Identify the nature of abnormal plasma lipoproteins described in biliary obstruction.





MATERIALS AND METHODS

Subjects. Fifty patients with suspected liver disease were studied using the combined facilities of the Yale-New Haven Medical Center and the National Heart Institute. Fasting plasma samples were obtained and the resulting lipoprotein profiles compared with alterations in hepatic function and histology. Several patients with acute disease were followed serially during the course of their hospitalization, others with chronic disease were evaluated at intervals of up to eight months. Those patients with concomitant thyroid disease, diabetes mellitus, pancreatitis, or other conditions known to alter plasma lipids were excluded from the study. The patients were classified as follows:

Acute hepatitis	17
Portal cirrhosis	13
Post-necrotic cirrhosis	3
Biliary obstruction	4
Biliary cirrhosis	11
primary	5
secondary	6
Normal liver	<u>2</u>
Total	50

In the seventeen patients with acute hepatitis (Table II) the diagnosis was based on the clinical picture and the biopsy findings. In fifteen patients these findings were most consistent with acute viral hepatitis, whereas in two, E.G. and R.B. there was a question of hepatocellular hepatitis secondary to drug hypersensitivity; cyclophosphamide (cytoxan) in the former, and halothane in the latter. The date of onset was arbitrarily defined as the day



on which jaundice or dark urine was first noted; this, however, was subject to certain inaccuracies since in many, vague symptoms of nausea, anorexia, or abdominal pain preceded the onset of icterus. All were jaundiced and had elevated serum glutamic oxaloacetic transaminase (SGOT) levels at the time of initial sampling. Four were followed serially during convalescence. One patient, E.B., developed acute hepatic necrosis and expired following unsuccessful attempts at exchange transfusions, however, the remainder survived with return of liver function values to normal.

In the thirteen patients with portal (Laennec's) cirrhosis (Table III), the typical histological changes of portal cirrhosis were found on liver biopsy and all patients gave a history of heavy alcoholic intake for more than 10 years. Ten patients were jaundiced at the time of sampling, and all were admitted for treatment of progressive hepatic deterioration with peripheral edema, ascites, and decreased serum albumin. Two patients, F.H. and N.T., had acute alcoholic hepatitis (florid cirrhosis) superimposed on pre-existing portal cirrhosis and were evaluated at weekly intervals.

Three patients with post-necrotic cirrhosis (Table IV) were studied. All were jaundiced and all were female, ranging in age from 38 (M.H.) to 76 (D.D.). In addition, two patients with hepatomegaly (Table IV) but histologically normal liver biopsy and normal liver function tests were evaluated.

Four patients with extra-hepatic biliary obstruction (Table IV) were studied. At the time of sampling, only one, J.C., was jaundiced with elevated serum alkaline phosphatase. One patient, M.H., was found on biopsy to have acute and chronic pericholangitis (etiology undetermined), while J.C., F.M., and D.G. later underwent cholecystectomy for choledocholithiasis and cholecystitis. All had histories of intermittent abdominal pain, pale stools and jaundice.



The diagnosis of biliary cirrhosis was made both on the basis of the liver biopsy and the clinical picture. The five with primary biliary cirrhosis (Table V-A) had histologically typical biopsies with exploratory laparotomies and operative cholangiograms that failed to reveal extra-hepatic sites of obstruction. All had hepatomegaly, hyperpigmentation, and a history of the insidious onset of jaundice with pruritis, darkening of the urine, and pale stools in the absence of abdominal pain, and chills and fever. Xanthelasma were present in all; R.I. and A.B. had tuberous and planar xanthomas, while C.D., M.D., and F.N. had a previous history of xanthomas. Ages ranged from 42 (F.N.) to 54 (C.D.) and all were female.

In the six patients with secondary biliary cirrhosis (Table V-B), four (T.L., J.C., T.W., and W.C.) had partial biliary atresia, W.W. had a history of neonatal hepatitis with biopsy proven biliary cirrhosis at age 2, and M.J. had chlorpromazine hydrochloride (Thorazine) induced cholestatic hepatitis documented by biopsy, with progression toward biliary cirrhosis. All had xanthelasma and, tuberous and planar xanthomas at the time of sampling with the exception of T.W. who was in a terminal state and expired within 4 weeks.

Sample Collection. Fasting blood samples were obtained from each patient following at 12-14 hour overnight fasting period. Samples were collected in ethylene-diaminotetra-acetic acid (EDTA), 1 mg/ml, and cooled to 2-4°C with subsequent separation of the plasma in a refrigerated centrifuge (2-4°C) at 2500 RPM. The supernatant plasma was refrigerated at 4°C until further tests were performed. Each sample was evaluated by means of paper electrophoresis, immunoelectrophoresis, and quantitative lipid analyses.



Paper Electrophoresis. Plasma lipoproteins were separated on paper electrophoresis by use of the modified Durrum hanging-strip method in barbital buffer of ionic strength 0.1, pH 8.6, containing 0.001 M EDTA and one per cent albumin (15). Electrophoresis was performed at room temperature for a 16 hour period at 120 volts and a current of 0.75 to 1.0 ma per strip using Whatman No. 1 paper. Following electrophoresis the strips were dried at 95°C for 20 minutes, then stained by immersion in a supersaturated alcoholic solution of Oil-red-O for 4-6 hours at 40°C, and finally rinsed with water and dried (16).

Precipitation. All the low density and very low density lipoproteins were separated from the plasma by polyanionic precipitation using heparin and manganese chloride in the cold. In this manner, alpha, or "high" density lipoproteins were isolated in supernatant, their cholesterol content being equivalent to that in the fraction of density greater than 1.063 isolated by preparative ultracentrifugation (29). Precipitation was accomplished by adding 0.15 ml 1.0 M manganese chloride and 6 mgm sodium heparin to 3 ml aliquots of plasma. The precipitate was allowed to develop for 15 minutes at 4°C and, following a 15 minute centrifugation at 4°C and 2,000 RPM, the supernatant was extracted.

Ultracentrifugation. Preparative ultracentrifugation was used to isolate the plasma lipoproteins employing the methods and salt solutions first described by Havel et al. (30). 5 ml aliquots of plasma were ultracentrifuged at their own density ( $D=1.006$ ) for 16 hours at  $100,000 \times g$  in the 40.3 rotor using the Spinco model L ultracentrifuge. The infranatant containing the fraction of density greater than 1.006 was then separated from the supernatant fraction





of density less than 1.006, using a lusteroid tube slicer and returned to a volume of 5 ml by the addition of 0.15 M-saline solution. The plasma fractions of density greater and less than 1.006 thus separated in the ultracentrifuge were then subjected to paper electrophoresis, and the mobility of their lipoprotein bands compared with each other and that of the lipoproteins in the whole plasma.

In several patients the plasma fraction of density greater than 1.006 was further separated in the ultracentrifuge into fractions of density greater than 1.063, and less than 1.063. This procedure provides a useful method for separating the normally occurring alpha and beta lipoproteins since the beta fraction is normally found in the density range 1.006 to 1.063, while the alpha fraction occurs in the density range 1.063 to 1.21. The density of the plasma was adjusted to 1.063 by the addition of KBr and NaCl. Ultracentrifugation was carried out in the 40.3 rotor for 16 hours at 2-4°C. The tubes were sliced and the density fractions further concentrated and purified by additional 16 hr periods of ultracentrifugation at 2-4°C using the 40.3 rotor. All fractions were then dialyzed at 4°C against 40 vol or more of 0.15 M NaCl containing 0.01 M EDTA at pH 6.5 to 7.3, the dialysis fluid being changed three times during a 24-hour period (29).

Lipid Analysis. Quantitative cholesterol determinations were performed on the plasma, the plasma fraction of density greater than 1.006, and the supernatant of the heparin-manganese chloride precipitation using the Technicon Autoanalyzer method (31). Plasma beta-lipoprotein cholesterol was expressed as the difference between the fraction of density greater than 1.006 and the supernatant of the heparin-manganese chloride precipitation (16). The plasma cholesterol less the cholesterol in density fraction



greater than 1.006 gave the normally occurring pre-beta and/or chylomicron cholesterol (16). Plasma glycerides were determined by use of the Technicon autoanalyzer method. Plasma phospholipids were determined, using the method of Stewart and Hendry (32).

Immuno-electrophoresis. Plasma immuno-electrophoresis was performed on each sample using both 1% agarose and 2% Noble agar employing the techniques of Grabar and Williams as modified for microscope slides by Scheidegger. The mobility of the lipoproteins is different on agar and agarose. In the agarose which is sulfate-free agar, beta lipoproteins move away from the origin assuming the mobility of beta-globulin and are more easily distinguished from prebeta and alpha lipoproteins. Immuno-electrophoresis was accomplished in 0.05 M barbital buffer at pH 8.2 using constant current (58 ma, agar, or 44 ma, agarose) for 40 minutes (33). Antisera (50 to 60  $\mu$ l) was then added to each trough and diffusion allowed to occur at room temperature (24-72 hours) in a high humidity chamber following which the slides were photographed, washed overnight with saline, then distilled water, and finally stained with Oil-red-O in 60% ethanol for 12 hours at 40°C. Several double diffusion studies in agar on Ouchterlony plates with absorption of antigens by specific antibodies were performed using standard techniques. The antisera were prepared and characterized as previously described by Levy and Fredrickson (29). Each sample was evaluated against anti-whole sera and, combined anti-alpha and beta lipoprotein sera (29). In addition, several samples were evaluated with specific anti-alpha, or anti-beta lipoprotein sera when indicated.

Delipidation Studies. Although ethanol-ether is known to precipitate



irreversibly the polypeptide of beta lipoprotein, it provides a relatively complete delipidation of alpha lipoprotein. In several patients this procedure was employed to further characterize lipoproteins which behaved immunochemically as alpha but presented with otherwise atypical properties. Plasma was injected through a no. 25 needle into 50 vol of ethanol:ether (3:2, vol:vol), and the mixture was left standing at 4°C for 18 to 24 hours. Following centrifugation at 4°C for 50-60 minutes, the precipitate was collected, washed once in ether, and dissolved in 1 ml of 0.9% NaCl (pH 7) containing 0.001 M EDTA. The samples were then subjected to a variety of immunochemical and electrophoretic studies.



## RESULTS

The Tables and Figures referred to in the following section are found in the Appendix. For purposes of comparison, the normal values for plasma lipid and lipoprotein concentrations are listed with normal liver function values in Table 1. The normal pattern on paper electrophoresis is demonstrated in Figure 9. The normal plasma immunoelectrophoretic pattern on agarose using combined anti-alpha and beta lipoprotein sera (29) is demonstrated in Figure 4. Using the same antisera, immunoelectrophoresis of normal plasma on agar results in a pattern similar to that of M.J. on 12/12/66, Figure 10, with the exception that the alpha band is less intense.

Acute Hepatitis. Lipids. The results of the plasma lipid determinations in seventeen patients with acute hepatitis are listed with their corresponding liver function values in Table II. Total cholesterol concentrations varied, being normal in eleven, depressed in four (E.B., R.B., M.W., and S.K), and elevated in two (A.R., and O.C). Plasma triglycerides were elevated in thirteen of seventeen, but only rarely to values over 250 mgm%.

Lipoproteins. Striking depressions of alpha lipoprotein concentration determined by heparin-manganese chloride precipitation were found in sixteen of the seventeen patients, and in the remainder, E.D., the value was in the lower range of normal. Alpha lipoproteins were also depressed or absent on paper electrophoresis and immunoelectrophoresis. In four subjects followed serially the depression of alpha lipoprotein concentration was reversible, returning to normal as liver function improved. This serial increase in





alpha lipoprotein values with recovery could be demonstrated by ultracentrifugation and precipitation as well as by paper electrophoresis (Figure 1) or immunoelectrophoresis (Figure 2). It should be noted in Figure 1 that the increasing alpha lipoprotein concentration, associated with improvement of liver function, was also accompanied by a return of lipid staining material in the prebeta region.

In contrast, beta lipoprotein concentrations were normal in sixteen of the seventeen. Only in O.C., where the total cholesterol was elevated to 565 mgm%, was the beta lipoprotein fraction elevated, this occurred in the presence of markedly depressed alpha lipoprotein concentrations. In those patients with elevated triglycerides and depressed alpha lipoprotein levels, material with beta mobility was atypically present at density less than 1.006 (Figure 3). With improvement of liver function and return of alpha lipoprotein concentrations to normal, lipid staining material in beta position could be found only in the density range 1.006 - 1.063 (normal beta lipoprotein density range) and the pre-beta band reappeared (Figure 1).

In the present study, no specific correlation was demonstrated between given liver function values and alpha lipoprotein concentration. However, in the four patients followed serially, improvement in liver function was associated with a return to normal alpha lipoprotein concentrations.

Portal Cirrhosis. Lipids. Plasma lipid and liver function values in thirteen with portal cirrhosis are listed in Table III. Total cholesterol varied, being normal in five, depressed in seven, and elevated in one. Plasma triglycerides were normal in nine and slightly elevated in four.

Lipoproteins. Moderate to severe decreases in alpha lipoprotein concentration were found in eleven of thirteen patients, the remainder, J.M.



and M.D., had values in the lower range of normal. As in acute hepatitis, alpha lipoproteins were depressed on paper electrophoresis, albeit not as severe, and similar changes were observed on immunoelectrophoresis. In two, F.H. and N.T., with acute hepatocellular disease superimposed on long standing cirrhosis there was a severe depression of alpha lipoproteins (Figure 4), similar to those seen in acute hepatitis. Beta lipoprotein concentrations were normal in ten of thirteen, being slightly depressed in two, F.J., and P.O., and elevated in one, M.D.

Post-necrotic Cirrhosis. The plasma lipids and liver function values in three patients with post-necrotic cirrhosis are given in Table IV. In all, the total cholesterol was decreased. All had decreased alpha lipoprotein concentrations by heparin-manganese chloride precipitation and by immunoelectrophoresis and paper electrophoresis. The beta lipoprotein concentrations were low normal.

Extra-hepatic Obstruction. The plasma lipids and liver function values in four patients with extra-hepatic obstruction are given in Table IV. Only one, J.C., had abnormal liver function values when sampled. None had elevated total cholesterols and the alpha lipoprotein concentrations were all within normal limits.

Normal liver biopsy. The plasma lipids and liver function values for two patients with normal liver biopsies are given in Table IV. Both were evaluated because of hepatomegaly, however, both had normal plasma lipid and liver function values. Normal patterns were present on paper electrophoresis and immunoelectrophoresis.

Biliary Cirrhosis. Lipids. Plasma lipids and liver function values



in eleven patients with biliary cirrhosis are presented in Table V. The patients have been divided into those with primary and those with secondary biliary cirrhosis, and listed in order based on the duration of disease. Total cholesterol varied in the eleven patients from 134 to 2,056 mgm%, being highest in those patients (M.J., and R.I.) whose disease was of the shortest duration. In seven patients of twelve, total cholesterols were elevated. In five on whom plasma phospholipids were determined, all were elevated, with decreases in the cholesterol-phospholipid ratio. In the entire group with biliary cirrhosis, the plasma triglycerides were normal or only slightly elevated and quite typically did not parallel the striking increases in cholesterol and phospholipids.

Lipoproteins. Unusual changes in alpha lipoprotein properties and concentrations were observed which varied with the degree of parenchymal damage and change in total lipids.

In five patients with late stage biliary cirrhosis and marked parenchymal destruction, the alpha lipoprotein concentrations were reduced as in hepatocellular disease. In one patient, F.N., alpha lipoprotein concentrations were observed to decrease in association with deterioration of the clinical picture and the appearance of Mallory bodies with continued parenchymal destruction on biopsy.

By contrast, in all seven patients with biliary obstruction and little or no evidence of parenchymal damage, the alpha lipoproteins were increased and showed unusual physical properties.

In five patients with moderately elevated cholesterols, (W.W., W.C., J.C., T.L., and M.J., 12.66) alpha lipoprotein concentration was increased, and a broad band of lipid staining material was found in the alpha position



on paper electrophoresis (Figure 5). Following ultracentrifugation, the lipid staining material with alpha lipoprotein mobility was present atypically at a density less than 1.063 (normally contains only beta-lipoprotein) as demonstrated both by paper electrophoresis (Figure 5) and immunoelectrophoresis (Figure 6). After precipitation of plasma with heparin-manganese chloride, the supernatant contained only material of alpha lipoprotein mobility. Following ultracentrifugation, substantial amounts of this material were found in the fraction of density less than 1.063 (Figure 7). The presence of material at a density less than 1.063 with alpha lipoprotein mobility on paper electrophoresis and immunoelectrophoresis was similarly demonstrated in the plasma supernatant following precipitation with specific anti-beta lipoprotein sera.

These results on immunoelectrophoresis and paper electrophoresis of the plasma fractions separated by ultracentrifugation and chemical precipitation, suggested the possibility of an abnormal lipoprotein form of beta lipoprotein density and alpha lipoprotein mobility. Isolated preparations of the abnormal lipoprotein fraction proved to have complete immunochemical identity with normal alpha lipoprotein. On immunodiffusion using agarose (Figure 8), the material with alpha lipoprotein mobility found at density less than 1.063, isolated in the supernatant either by heparin-manganese chloride precipitation or addition of specific anti-beta lipoprotein sera, proved to have complete immunochemical identity with alpha lipoprotein obtained from a normal subject.

In two patients, R.I. and M.J., 9.66, with minimal parenchymal damage, total cholesterol exceeded 1,000 mgm%. Alpha lipoproteins were not detected by heparin-manganese chloride precipitation, however an unusual pattern was encountered on both paper and immunoelectrophoresis. On paper electrophoresis,





(Figure 9, M.M - 9/15/66) normal lipid staining material was present in the beta position, none appeared in the alpha position, and a peculiar, thin band of cathode-migrating, lipid staining material was present near the origin. Similarly, following immunoelectrophoresis on agar (Figure 10, M.J. - 9/15/66) using combined anti-alpha and beta lipoprotein sera, two immunochemically distinct lipoprotein fractions were identified, one in the normal beta position, the other encircling the well and migrating behind the lipoprotein fraction in beta position. On ultracentrifugation essentially all of the lipoproteins were isolated between density 1.006 and 1.063 (Figure 11).

Several procedures were undertaken to immunochemically define the two lipoprotein fractions, and representative patterns following immunoelectrophoresis on agar are given in Figure 11. Agar was used for immunoelectrophoresis rather than agarose, since it provided better separation of the two lipoprotein fractions. The lipoprotein fraction migrating in beta position was not present (Figure 11, plate A) when: (1) the plasma was precipitated with anti-beta lipoprotein sera before immunoelectrophoresis, (2) the combined anti-alpha and beta lipoprotein sera was absorbed with beta lipoprotein, or (3) specific anti-alpha lipoprotein sera was used in immunoelectrophoresis rather than the combined anti-alpha and beta lipoprotein sera. Similarly, the lipoprotein fraction encircling the well and migrating behind the lipoprotein fraction in beta position was not present (Figure 11, plate B) when (1) the plasma was precipitated with anti-alpha lipoprotein sera before immunoelectrophoresis, (2) the combined anti-alpha and beta lipoprotein sera was absorbed with alpha lipoprotein, or (3) specific anti-beta lipoprotein sera was used in immunoelectrophoresis rather than the combined anti-alpha and beta lipoprotein sera.



When the atypical lipoprotein fraction migrating near the beta lipoprotein fraction was delipidated using ethanol-ether, the resulting peptide was found to have alpha mobility on immunoelectrophoresis using agarose, and reacted with specific anti-alpha lipoprotein sera (Figure 12, plate A), but not with specific anti-beta lipoprotein sera (Figure 12, plate B).

The two lipoprotein fractions occurring at density less than 1.063 with beta lipoprotein mobility on immunoelectrophoresis were thus defined immunochemically as beta and alpha lipoprotein, the latter assuming alpha mobility when delipidated.

One patient, M.J., with cholestatic hepatitis was followed during a three month period as total cholesterol concentration fell from 2056 mgm% to 668 mgm%. Initially, when total cholesterol concentrations exceeded 2056 mgm%, the electrophoretic mobility of alpha lipoprotein was altered as previously described (Figure 9, M.J., 9.15 and Figure 10, M.J., 9.15). However, as total cholesterol concentration decreased to 668 mgm% alpha lipoproteins regained their normal mobility on paper electrophoresis (Figure 9, M.J., 12.12), where they were present as a broad, intensely staining band. A similar return of alpha lipoproteins to normal position was observed on immunoelectrophoresis in agar (Figure 10, M.J., 12.12). As total cholesterol concentration decreased, alpha lipoprotein concentration remained elevated, and although alpha lipoproteins were present in the supernatant following heparin-manganese chloride precipitation, large percentages were found at densities less than 1.063.



## DISCUSSION

The present study demonstrates the alterations in normal plasma lipoprotein patterns which occur in liver disease. The patterns for hepatocellular disease and biliary obstruction are different. In hepatocellular disease, plasma alpha lipoprotein concentration is reduced. In biliary obstruction unaccompanied by hepatocellular destruction, the concentration of plasma alpha lipoprotein is increased and its ultracentrifugal and electrophoretic properties are altered. The previously described abnormal lipoproteins of biliary obstruction are seen to result from changes in the properties and concentration of normal alpha lipoprotein.

The reduction of plasma alpha lipoproteins in acute hepatitis is striking, and the plasma lipoprotein patterns on immunoelectrophoresis and paper electrophoresis are difficult to distinguish from those of Tangier disease (16,34), in which plasma alpha lipoproteins are congenitally absent or severely deficient. In acute hepatitis, material with beta mobility is atypically present at densities less than 1.006 when hyperglyceridemia accompanies the severely depressed alpha lipoprotein concentrations. Similar patterns are found in Tangier disease when hyperglyceridemia is induced with high carbohydrate diets (35). As alpha lipoprotein concentration returns to normal, the glycerides are once again transported in the prebeta fraction. No evidence was obtained in the present study to suggest that alpha lipoprotein deficiencies in hepatocellular disease are secondary to hyperglyceridemia.

Deficiencies of plasma alpha lipoproteins in hepatocellular disease



have previously been reported by Pierce and Gofman (24), and later by Eder et al. (2) who observed reversible depression of plasma alpha lipoproteins on Cohn Fractionation of patients followed serially with acute hepatitis. No specific correlation with liver function tests was noted by Eder et al., however, return to normal alpha lipoprotein patterns coincided with subsidence of icterus. Pierce et al. (22) have correlated the lipoprotein patterns in acute hepatitis with changes in the icterus index and total bilirubin concentrations. In the present study no specific correlations between plasma alpha lipoprotein concentrations and liver function tests were noted, however, in acute hepatitis, improvement in liver function values was associated with rising alpha lipoprotein concentration.

In biliary obstruction with hepatocellular damage, the alpha lipoprotein levels are low as in other forms of hepatocellular disease, and the lipids are transported by the beta lipoproteins. The reasons for decreased plasma alpha lipoprotein levels in hepatocellular disease are unestablished. The liver has been demonstrated as a site of lipoprotein synthesis in perfused rat liver (36-38). However, extra-hepatic sites of lipoprotein synthesis have been demonstrated (39-40), and recent studies indicate lipoprotein synthesis continues following hepatectomy in the dog (41). Both plasma lipoproteins have a biological half-life of about four days as determined by  $I^{131}$  studies (42,43). Thus, from data currently available, there is no apparent explanation for the preferential effect of hepatocellular disease on plasma alpha lipoprotein concentration.

This study demonstrates that increases in alpha lipoprotein concen-





tration result when biliary obstruction occurs with only minimal hepatocellular destruction. With increasing hyperlipidemia the electrophoretic and ultracentrifugal properties are remarkably altered resulting in the appearance of an abnormal lipoprotein. Lemaire et al. (26,27) have observed similar alterations in obstructive jaundice. They describe a lipoprotein form with antigenicity close to that of alpha and immunoelectrophoretic mobility and ultracentrifugal characteristics similar to that of the beta lipoproteins. The present study demonstrates the immunochemical identity of alpha lipoproteins from normal plasma with the abnormal lipoproteins appearing at plasma densities less than 1.063 in obstructive jaundice.

The effects of biliary obstruction on the plasma lipoprotein pattern are unusual. Considerable debate has surrounded the nature of the abnormal lipoproteins in this condition. Even though plasma lipoproteins may be characterized by a variety of methods which include electrophoresis, ultracentrifugation, and chemical and immunochemical precipitation, none of these provides an absolute method of classification. This is demonstrated by the paradoxical nature of the abnormal lipoproteins previously described in obstructive jaundice (2,5) which behave as alpha lipoproteins on Cohn fractionation but resemble beta lipoproteins in their ultracentrifugal and electrophoretic patterns.

Recently, Fredrickson and Levy (16,28,29) have stressed the value of immunoelectrophoresis in the characterization of plasma lipoproteins. The method permits identification of the lipoprotein apoproteins, the protein portion, as either alpha or beta, and is relatively specific for the peptide moiety, albeit ultracentrifugation, thawing, and storage at room temperature may slightly alter alpha lipoproteins (16) and minor genetic variations



in beta lipoproteins have been reported (28). Hence, the definition of lipoproteins as abnormal must appropriately include antigenic characterization.

The present study confirms the value of immunochemical techniques in the interpretation of plasma lipoprotein function and identity. In states of hyperphospholipidemia and hypercholesterolemia the density and electrophoretic mobility of the plasma lipoproteins are altered without changing their antigenicity. In this situation, identification of the peptide moiety provides the clue to a more specific understanding of the altered plasma lipoprotein patterns.

The mechanism by which alpha lipoproteins are altered in biliary obstruction remains unestablished. Lemaire et al. (26,27) have recently shown that plasma phospholipids in obstructive jaundice are trichlorethylene extractable whereas in normals and those with hepatocellular disease this is not the case. Moreover, the phospholipids are only extracted from the "abnormal lipoproteins" in obstructive jaundice. They postulate a disruption of the normal protein phospholipid bond to occur in obstructive jaundice, which thereby alters the stability and properties of the lipoprotein. Lecithin and free cholesterol are known to be the major lipids present in bile (44), however, the mechanism of the hyperphospholipidemia and hypercholesterolemia in biliary obstruction is poorly understood. Retention of bile lipids does not appear to account entirely for the hyperlipidemia, since an increased rate of cholesterol synthesis is observed following bile duct ligation in the rat (45) and phospholipid synthesis is increased in patients with jaundice and hyperlipidemia (46). Byers et al. (47) have noted hypercholestermia occurring in rats eight hours following



intravenous lecithin infusion, however, increased C<sup>14</sup> labeled acetate uptake was not observed until 24 hours following the infusion. They conclude that hyperphospholipidemia causes a shift in cholesterol from peripheral tissues to blood thereby altering the partition of cholesterol between liver and blood and indirectly resulting in net increase of cholesterol synthesis. The effects of hyperphospholipidemia on lipoprotein synthesis are unestablished. Similar alterations in the ultracentrifugal properties of alpha lipoprotein are reported in abeta lipoproteinemia (48).

The metabolism and transport kinetics of plasma lipoproteins are not well understood. The present study demonstrates a pronounced effect of hepatocellular disease and biliary obstruction on plasma alpha lipoprotein concentration. These variations may prove of value diagnostically in the differentiation of obstructive and hepatocellular jaundice. Of theoretical as well as practical interest is the role of biliary obstruction and the associated hyperphospholipidemia and hypercholesterolemia in altering the properties and concentrations of plasma alpha lipoprotein. Future investigations in this field using this abnormal lipoprotein as a model will undoubtedly enable us to gain a more precise understanding of the relationship of lipid to protein in the complex lipoproteins.

The demonstration of an acquired plasma lipoprotein deficiency in hepatocellular disease is noteworthy and emphasizes the essential role of the liver in the overall metabolism of plasma lipids and lipoproteins.



SUMMARY

Lipoprotein profiles from fifty patients with liver disease were compared to alterations in hepatic function and histology. The effects of liver disease were reflected by either an increase or depression of plasma alpha lipoprotein concentration. Increases were accompanied by unusual changes in the properties of these lipoproteins.

Alpha lipoproteins were absent on paper electrophoresis of plasma from two biliary cirrhotics (one primary) with minimal parenchymal damage and cholesterol > 1,000 mg%, however, two immunochemically different Heparin-MnCl<sub>2</sub> precipitable lipoproteins were found between densities 1.006 - 1.063 which migrated slowly on agar and agarose. One lipoprotein was beta; the other reacted with anti-alpha lipoprotein sera and, when delipidated assumed alpha mobility. In five biliary cirrhotics with moderately elevated lipids, alpha lipoproteins were increased and again found between densities 1.006-1.063, however, in these, the alpha lipoproteins formed a broad alpha migrating band on paper and agar electrophoresis. Five late stage biliary cirrhotics had markedly depressed alpha lipoprotein levels similar to those encountered in seventeen patients with acute hepatitis. In four of the latter followed serially, alpha lipoproteins returned to normal levels as hepatic function improved. In thirteen with Laennec's cirrhosis and three with post-necrotic cirrhosis alpha lipoproteins were depressed.

It is suggested that biliary obstruction without hepatocellular destruction alters the properties of alpha lipoprotein. With hepatocellular destruction, alpha lipoprotein levels are decreased. These observations are of theoretical and diagnostic interest.





BIBLIOGRAPHY

1. Phillips, G.B. The lipid composition of serum in patients with liver disease. *J. Clin. Invest.* 1960, 39, 1639.
2. Eder, H.A., Russ, E.M., Pritchett, R.A., Wilber, M.M., and Barr, D.P. Protein-lipid relationships in human plasma: In biliary cirrhosis, obstructive jaundice and acute hepatitis. *J. Clin. Invest.* 1955, 34, 1147.
3. Addison, T., and Gull, W. On a certain affection of the skin, Vitiligoidea- $\alpha$ .Plana,  $\beta$ .Tuberosa. *Guy's Hosp. Repts.*, 1851, 7, 265.
4. Flint, A., Jr. Experimental researches into a new excretory function of the liver; consisting in the removal of cholesterine from the blood, and its discharge from the body in the form of stercorine. *Amer. J. Med. Sci.* 1862, 44, 305.
5. Russ, E.M., Raymunt, J., and Barr, D.P. Lipoproteins in primary biliary cirrhosis. *J. Clin. Invest.* 1956, 35, 133.
6. Man, E.B., Kartin, B.L., Durlacher, S.H., and Peters, J.P. The lipids of serum and liver in patients with hepatic diseases. *J. Clin. Invest.* 1945, 24, 623.
7. Albrink, M.J., Man, E.B., and Peters, J.P. Serum lipids in infectious hepatitis and obstructive jaundice. *J. Clin. Invest.* 1950, 29, 781.
8. Epstein, E.Z. Cholesterol of the blood plasma in hepatic and biliary diseases. *Arch. Intern. Med.* 1932, 50, 203.
9. Ahrens, E.H., and Kunkel, H.G. The relationship between serum lipids and skin xanthomata in eighteen patients with primary biliary cirrhosis. *J. Clin. Invest.* 1949, 28, 1565.
10. Petersen, V.P. The individual plasma phospholipids in obstructive jaundice. *Acta. Med. Scand.* 1953, 144, 345.



11. Albrink, M.J. The choline-containing phospholipids of serum. *J. Clin. Invest.* 1950, 29, 46.
12. Mendenhall, C.L., and Mortiaux, M.D. Alterations in serum triglyceride levels in liver disease. *Gastroent.* 1962, 42, 684.
13. Post, J., Gellis, S., and Lindenauer, H.J. Studies on the sequelae of acute infectious hepatitis. *Ann. Int. Med.* 1950, 33, 1378.
14. Petersen, V.P. The individual plasma phospholipids in acute hepatitis. *Acta. Med. Scand.* 1953, 144, 345.
15. Lees, R.S., and Hatch, F.T. Sharper separation of lipoprotein species by paper electrophoresis in albumin-containing buffer. *J. Lab. & Clin. Med.* 1963, 61, 518.
16. Fredrickson, D.S., Levy, R.I., and Lees, R.S. Fat transport in lipoproteins—an integrated approach to mechanisms and disorders. *New Eng. J. Med.* 1967, 276, 34.
17. Cohn, E.J., Gurd, F.R.N., Surgenor, D.M., Barnes, B.A., Brown, R.K., Derovaux, G., Gillespie, J.M., Kahnt, F.W., Lever, W.F., Liu, C.H., Mittelman, D., Mouton, R.F., Schmid, K., and Uroma, E. A system for the separation of the components of human blood: quantitative procedures for the separation of the protein components of human plasma. *J. Am. Chem. Soc.* 1950, 72, 465.
18. Furman, R.H., and Conrad, L. L. Ultracentrifugal characterization of the lipoprotein spectrum in obstructive jaundice: Studies of serum lipid relationships in intra- and extrahepatic biliary obstruction. *J. Clin. Invest.* 1957, 36, 713.
19. Gofman, J.W., DeLalla, O., Glazier, F., Freeman, N.K., Lindgren, F.T.



- Nichols, A.V., Strisower, B., and Tamplin, A.R. The serum lipoprotein transport system in health, metabolic disorders, atherosclerosis, and coronary heart disease. *Plasma*. 1954, 2, 413.
20. Kunkel, H.G., and Slater, R.J. Lipoprotein patterns of serum obtained by zone electrophoresis. *J. Clin. Invest.* 1952, 31, 677.
21. Kunkel, H.G., and Ahrens, H.H. The relationship between serum lipids and the electrophoretic pattern, with particular reference to patients with primary biliary cirrhosis. *J. Clin. Invest.* 1948, 28, 1575.
22. Pierce, F.T., Kimmel, J.R., and Burns, T.W. Lipoproteins in infectious and serum hepatitis. *Metab.* 1954, 3, 228.
23. McGinley, J., Jones, H., and Gofman, J. Lipoproteins and xanthomatous diseases. *J. Invest. Derm.* 1952, 19, 71.
24. Pierce, F.T., and Gofman, J.W., Lipoproteins, liver disease, and atherosclerosis. *Circulation* 1951, 4, 25.
25. DeLalla, L., Levine, L., and Brown, R.K. Immunologic studies of human high density lipoproteins. *J. Exp. Med.* 1957, 106, 261.
26. Lemaire, A., Etienne, J., Polonovski, J., Housset, E., and Cottet, J. Un Nouveau test biologique de la cholostase. *La Presse Med.* 1965, 73, 3245.
27. Etienne, J., Lemaire, A., Cottet, J., and Loeper, J. Test du papier au trichlorethylene etude de la fragilite lipoproteinique. *Rev. Int. Hepat.* 1966, 16, 169.
28. Fredrickson, D.S., and Levy, R.I. A functional view of the plasma lipoproteins. In Proceedings of the Mosbach Colloquium of the Society for Physiological Chemistry. Heidelberg: Springer (in press).



29. Levy, R.I., and Fredrickson, D.S. Heterogeneity of plasma high density lipoproteins. *J. Clin. Invest.* 1965, 44, 426.
30. Havel, R.J., Eder, H.A., and Bragdon, J.H. The distribution and chemical composition of ultracentrifugally separated lipoproteins in human serum. *J. Clin. Invest.* 1955, 34, 1345.
31. Total cholesterol procedure N-24. In Auto Analyzer Manual. Chauncey, New York, Technicon Instruments Corp., 1964.
32. Stewart, C.P., and Hendry, E.B. The phospholipins of blood. *Biochem. J.* 1935, 29, 1683.
33. Scheidegger, J.J. Une micro-methode de l'immuno-electrophorese. *Int. Arch. Allergy* 1955, 7, 103.
34. Fredrickson, D.S. Familial high density lipoprotein deficiency: Tangier disease. In The Metabolic Basis of Inherited Disease. Second Edition. Edited by J.B. Stanbury, J.B. Wyngaarden and D. S. Fredrickson. 1434 pp. New York: McGraw-Hill, 1966, p 486.
35. Levy, R.I., Lees, R.S., and Fredrickson, D.S. Nature of prebeta (very low density) lipoproteins. *J. Clin. Invest.* 1966, 45, 63.
36. Radding, C.M., Bragdon, J.H., and Steinberg, D. Synthesis of low- and high-density lipoproteins by rat liver in vitro. *Biochem. et Biophys. Acta* 1958, 30, 443.
37. Marsh, J.B., and Whereat, A.F. Synthesis of plasma lipoproteins by rat liver. *J. Biol. Chem.* 1959, 234, 3196.
38. Haft, D.E., Roheim, P.S., White, A., and Eder, H.A. Plasma lipoprotein metabolism in perfused rat livers. I. Protein synthesis and entry into plasma. *J. Clin. Invest.* 1962, 41, 842.





39. Rodbell, M., Fredrickson, D.S., and Ono, K. Metabolism of chylomicron proteins in dog. *J. Biol. Chem.* 1959, 234, 567.
40. Hatch, F.T., Hagopian, L.M., Rubinstein, J.J., and Canellos, G.P. Incorporation of labeled leucine into lipoprotein protein by rat intestinal mucosa. *Circulation* 1963, 28, 659.
41. Roheim, P.S., Gidez, L.I., and Eder, H.A. Extrahepatic synthesis of lipoproteins of plasma and chyle: role of intestine. *J. Clin. Invest.* 1966, 45, 297.
42. Gitlin, D. Studies on metabolism of plasma proteins in nephrotic syndrome. II. Lipoproteins. *J. Clin. Invest.* 1958, 37, 172.
43. Volwiler, W. Biosynthetic determination with radioactive sulfur of turnover rates of various plasma proteins in normal and cirrhotic man. *J. Clin. Invest.* 1955, 34, 1126.
44. Phillips, G.B. The lipid composition of human bile. *Biochim. Biophys. Acta.* 1960, 41, 361.
45. Fredrickson, D.S., Loud, A.V., Hinkelman, B.T., Schneider, H.S., and Frantz, I.D., Jr. The effect of ligation of the common bile duct on cholesterol synthesis in the rat. *J. Exp. Med.* 1954, 99, 43.
46. Balfour, W.M. Human plasma phospholipid formation: A study made with the aid of radiophosphorus. *Gastroent.* 1947, 9, 686.
47. Byers, S.O., Friedman, M., and Sugiyama, T. Mechanism underlying phosphatide-induced hypercholesterolemia. *J. Biol. Chem.* 1962, 237, 3375.
48. Levy, R.I., Fredrickson, D.S., and Laster, L. The lipoproteins and lipid transport in abetalipoproteinemia. *J. Clin. Invest.* 1966, 45, 531.
49. Smith, S.C., Scheig, R.L., Klatskin, G., and Levy, R.I. Lipoprotein abnormalities in liver disease. (Abstract) *Clin. Res.* 1967, 15, 330.



APPENDIX



TABLE I

Normal Values for plasma lipids and liver function tests.

A. Plasma Lipids\*

<u>Age Years</u>	<u>Cholesterol</u>		<u>Beta</u> mg/100 ml.	<u>Triglyceride</u> mg/100 ml
	<u>Total</u> mg/100 ml.	<u>Alpha</u> Male mg/100 ml.      Female mg/100 ml.		
0-19	120-230	30-65      30-70	50-170	10-140
20-29	120-240	35-70      30-75	60-170	10-140
30-39	140-270	30-65      35-80	70-190	10-150
40-49	150-310	30-65      40-85	80-190	10-160
50-59	160-330	30-65      35-85	80-210	10-190

B. Plasma Phospholipids\*\*

<u>Age Years</u>	<u>Phospholipids, mean and 90 per cent limits</u>
1-19	215(155-265)
20-29	235(175-300)
30-39	270(205-340)
40-49	275(185-355)
50-59	305(225-380)

C. Normal liver function values

Bilirubin.	Total	less than	1.30 mg/100 ml.
	Direct	less than	0.30 mg/100 ml.
Alkaline Phosphatase less than 8.6 Shinowara, J.R. et al. units			
Serum glutamic oxaloacetic transaminase (SGOT) 15-41 units			

\* Based on 95 per cent fiducial limits calculated for small samples-all values rounded to nearest 5 mg. Taken from Fredrickson, Levy and Lees, New England J. Med, 1967, 276, 34.

\*\* From Stanbury, Wyngaarden and Fredrickson, The Metabolic Basis of Inherited



TABLE II

Plasma lipid and liver function values  
in seventeen patients with acute hepatitis\*

Subject	Age	Sex	Day	Cholesterol				PL	Bilirubin Tot./Direct	Alk. Phos.	SGOT
				Total	Alpha	Beta	Trig.				
F.M.	43	M	16	150	5	117	239	336	20.0/12.0	7.2	906
			23	184	13	145	188	262	13.6/6.6	8.9	234
			30	186	29	117	182	248	5.2/2.7	6.3	112
			45		40			386	2.2/1.0	6.1	25
H.R.	24	F	16	172	31	126	134		8.0/5.0	5.2	750
			23	182	42	134	77	226	5.1/3.2	5.3	122
			30	188	51	119	75	243			
R.L.	27	M	4	190	18	144	297		17.0/10.0	7.4	980
			11	174	27	133	134				
M.D.	59	F	21	282	28	182	273	298	3.4/1.6	9.7	119
			35	258	33			265		7.0	36
E.B.	50	F	2	153	6	124	60	137	19.7/5.7	21.3	1296
J.K.	20	M	9	174	6	142	174		4.1/2.0	5.6	112
E.G.	59	M	31	228	10	162	296		8.0/4.0	5.5	51
R.B.	57	F	44	114	6	104	166	157	70.0/38.0	16.8	365
A.R.	23	M	14	271	28		177		8.0/5.0	14.9	238
P.C.	18	M	22	132	16	84	163		6.2/3.1	12.5	572
M.W.	62	F	12	112	6	100			32.0/15.0	17.1	237
F.W.	49	M	28	220	9	187			3.0/2.1	3.8	64
A.C.	40	M	28	230	16	178	298		4.0/2.0	24.5	480
W.F.	18	M	16	194	19	155	174		6.0/3.0	14.1	301
E.D.	20	M	17	178	36	124	100		2.0/1.0	8.4	153
O.C.	64	F	19	565	15	325	534		15.0/6.0	15.0	310
S.K.	22	M	6	110	3	75	224		2.0/1.0	3.2	430

\* Abbreviations: Trig.= Triglyceride, PL= Phospholipid, Alk. Phos.= Alkaline Phosphatase, SGOT= Serum glutamic oxaloacetic transaminase.





TABLE III

Plasma lipid and liver function values  
in thirteen patients with portal cirrhosis\*

Subject	Age	Sex	Cholesterol				PL	Bilirubin Tot./Dir.	Alk. Phos.	SGOT	Proteins Alb./Glob.
			Total	Alpha	Beta	Trig.					
			mg/100 ml.								
M.C.	64	F	166	17	121	150		1.6/0.7	2.2	35	3.0/3.2
A.L.	39	M	160	16	102	160	199	0.8/0.4	5.6	45	2.1/4.8
P.O.	68	M	104	27	67	20	113	2.0/0.8	7.5	57	1.8/4.0
J.M.	46	M	162	36	110	34		6.0/3.5	3.8	107	1.7/6.3
M.D.	49	F	350	32	290	166	293	3.0/1.0	7.2	59	3.4/3.3
F.M.	52	M	102	4	86	85		21.0/16.0	8.6	255	2.6/5.1
			124	8	106	68	140	20.1/11.2	8.6	164	
			142	10	126	29		9.0/4.5	5.9	144	2.6/5.5
N.T.	51	M	128	3	102	200	214	35.1/25.2	19.3	425	2.2/4.2
			180	6			290	51.2/30.2	8.1	250	
			184	8	138	133	250	14.1/8.0	6.3	168	2.3/3.7
A.P.	67	F	146	24	98	117	177	1.1/0.3	6.5	43	2.6/5.1
H.D.	44	F	122	15	95	98		9.1/5.7	7.7	82	2.7/3.5
J.B.	27	F	198	30	128	294		7.6/4.2	7.7	88	2.4/5.9
S.B.	70	F	110	5	105	89		16.2/8.1	16.7	147	2.3/4.7
F.J.	47	M	84	16	62	50		0.7/0.1	9.8	32	2.6/3.9
M.J.	51	F	178	17	133	128		1.5/0.7	16.8	44	2.6/4.4

\* Abbreviations: Trig.= Triglyceride, PL= Phospholipid, Alk. Phos.= Alkaline Phosphatase, SGOT= Serum glutamic oxaloacetic transaminase, Tot.= total, Dir.= direct, Alb.= Albumin, Glob.= globulin.



TABLE IV

Plasma lipid and liver function values in other patients studied\*

Subject	age	sex	Cholesterol					PL	Bilirubin Tot./Dir.	Alk. Phos.	SGOT
			Total	Alpha	Beta	Trig.	mg/100 ml.				
A. Post-necrotic cirrhosis											
R.S.	65	F	136	28	104			4.2/1.4	13	68	
D.D.	76	F	128	23	77	158	204	2.1/0.7	4	13	
M.H.	38	F	160	29	107	70		3.2/1.6	27	68	
B. Extra-hepatic obstruction											
J.C.	76	M	280	30	232	134	302	3.0/1.7	13	81	
F.M.	23	F	120	35	77	65		0.7/0.3	7	54	
D.G.	45	M	158	40	116	54		1.1/0.5	5	31	
M.H.	43	F	226	37	153	230		0.9/0.2	9	64	
C. Normal liver biopsy											
R.R.	71	F	170	34	124	81		0.4/0.1	4.7	11	
L.A.	65	F	198	45	125	136		0.7/0.1	3.4	11	

\* Abbreviations: Trig.= Triglyceride, PL= Phospholipid, Alk. Phos.= Alkaline phosphatase, SGOT= Serum glutamic oxaloacetic transaminase, Tot.= Total, Dir.= Direct.



TABLE V

Plasma lipid and liver function values  
in eleven patients with biliary cirrhosis\*

Subject	Age	Sex	Duration years	Date	Cholesterol				PL	Bilirubin Tot./Dir.	Alk. Phos.	SGOT
					Total	Alpha	Beta	Trig.				
A. Primary biliary cirrhosis												
R. I.	45	F	2	8/66	965	N.P. §	908	408		8.5/4.5	44	176
				1/67	1140	N.P. §	1096	348		10.0/4.0	103	112
C. D.	54	F	4	8/66	150	5	129	143		42.3/23.1	21	112
				10/66	238	7	231	126		24.0/12.4	21	85
A. B.	51	F	5	1/66	452	10	406	282	809	20.1/12.3	63	114
				8/66	506	7	463	290		30.2/18.3	63	51
M. D.	46	F	5	1/66	292	40	222	92	383	4.2/2.0	43	222
F. N.	42	F	6	2/66	312	34	246	135		4.4/2.3	60	175
				8/66	404	13	373	190		758	13.2/6.1	38
B. Secondary biliary cirrhosis												
M. J.	19	M	1/2	9/66	2056	N.P.	2000	221		22.0/13.4	235	195
				12/66	668	82	534	114		2.0/1.0	197	110
T. L.	6	M	6	11/66	236	106	94	99		1.0/0.1	40	141
J. C.	9	F	9	5/66	192	60	130	59	279	5.0/3.2	25	256
W. C.	9	F	9	7/65	364	128	225	92	444	1.2/0.5	65	108
W. W.	13	F	12	7/66	276	96	178	86		1.0/0.3	64	122
T. W.	18	M	18	12/66	134	6	110	182		9.2/4.3	16	42

\* Abbreviations: Trig.= Triglyceride, PL= Phospholipid, Alk. Phos.= Alkaline phosphatase, SGOT= Serum glutamic oxaloacetic transaminase, Tot.= Total, Dir.= Direct.

§ N.P.= none present following precipitation with heparin-MnCl<sub>2</sub>.



FIGURE 1.

SERIAL LIPOPROTEIN ELECTROPHORESIS ON PAPER OF PLASMA SAMPLES  
FROM PATIENT F.M. RECOVERING FROM ACUTE VIRAL HEPATITIS.

The alpha and prebeta lipoprotein bands are seen to return  
with improving liver function values. The proteins are  
stained for lipid with oil red O after electrophoresis.  
All paper strips subsequently shown stained with oil red O.





HEPATITIS

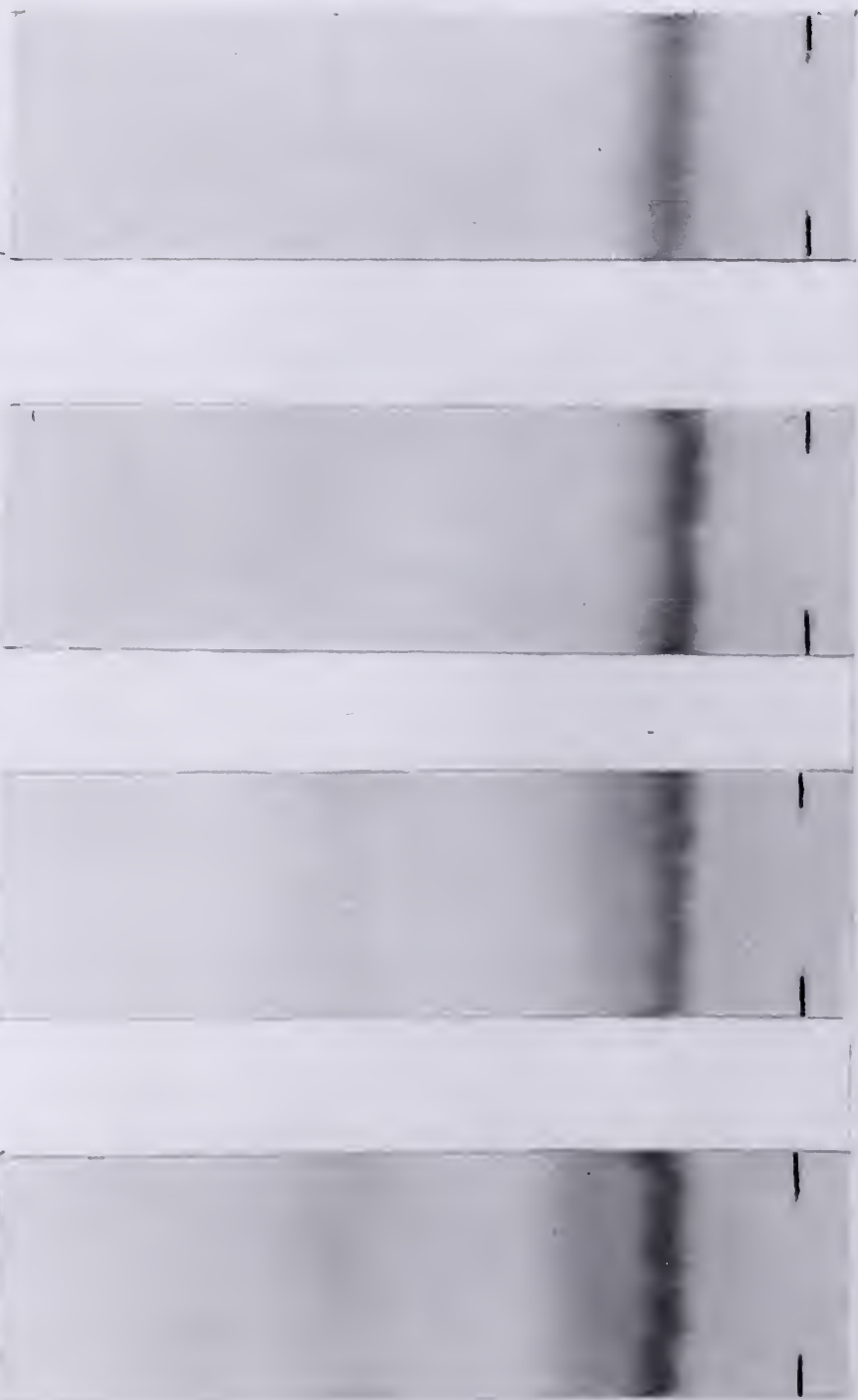
F. M.  
43 y. o. w ♂

ORIGIN

BETA

PREBETA

ALPHA



4/6

4/13

4/20

5/5

α CHOL

5

13

29

40

TRIG

239

188

182

220

SGOT

906

234

112

25



FIGURE 2.

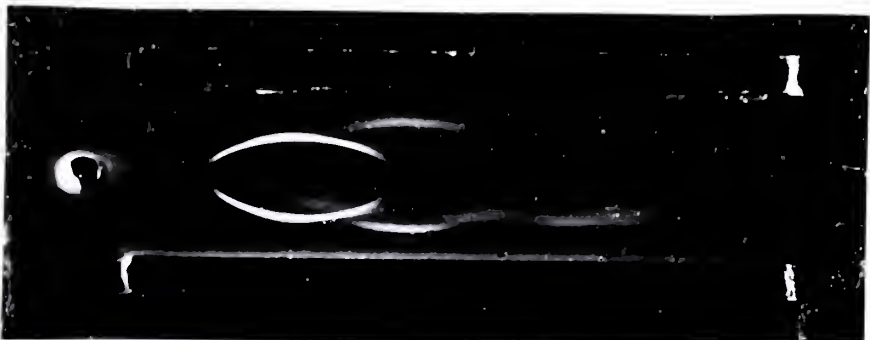
SERIAL IMMUNOELECTROPHORESIS ON AGAROSE OF PLASMA FROM  
PATIENT F.M. RECOVERING FROM ACUTE VIRAL HEPATITIS.

The troughs contain combined anti-alpha and beta  
lipoprotein sera R<sub>3</sub>A (48). All visible precipi-  
tation lines were shown to stain for lipid.

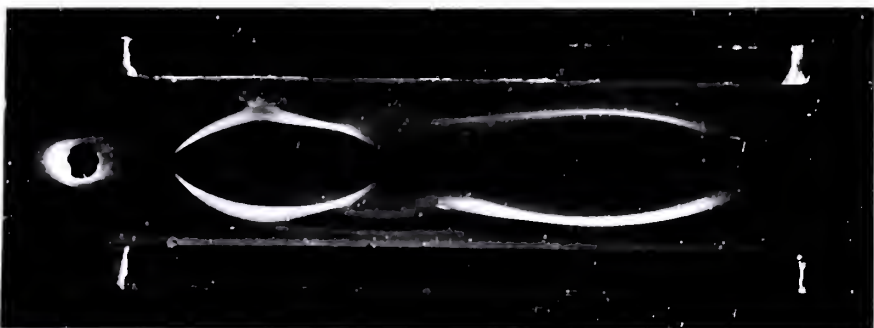


HEPATITIS

F. M.  
13 Y.O. W.M.



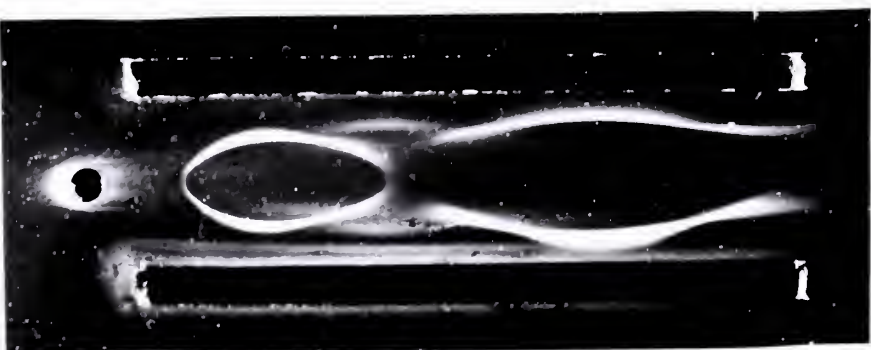
4/6



4/13



4/20



5/5



FIGURE 3.

PAPER ELECTROPHORESIS OF PLASMA FROM A PATIENT WITH ACUTE VIRAL HEPATITIS AND ELEVATED PLASMA TRIGLYCERIDES.

Lipid staining material is atypically present in the beta position at  $D < 1.006$ . The alpha lipoprotein concentration is severely depressed and no alpha lipoprotein band is present on paper electrophoresis. With return of alpha lipoprotein concentrations to normal, the lipid staining material at  $D < 1.006$  was found in the normal prebeta position.





ORIGIN

BETA

PREBETA

ALPHA

PLASMA

DENSITY < 1.006

DENSITY > 1.006

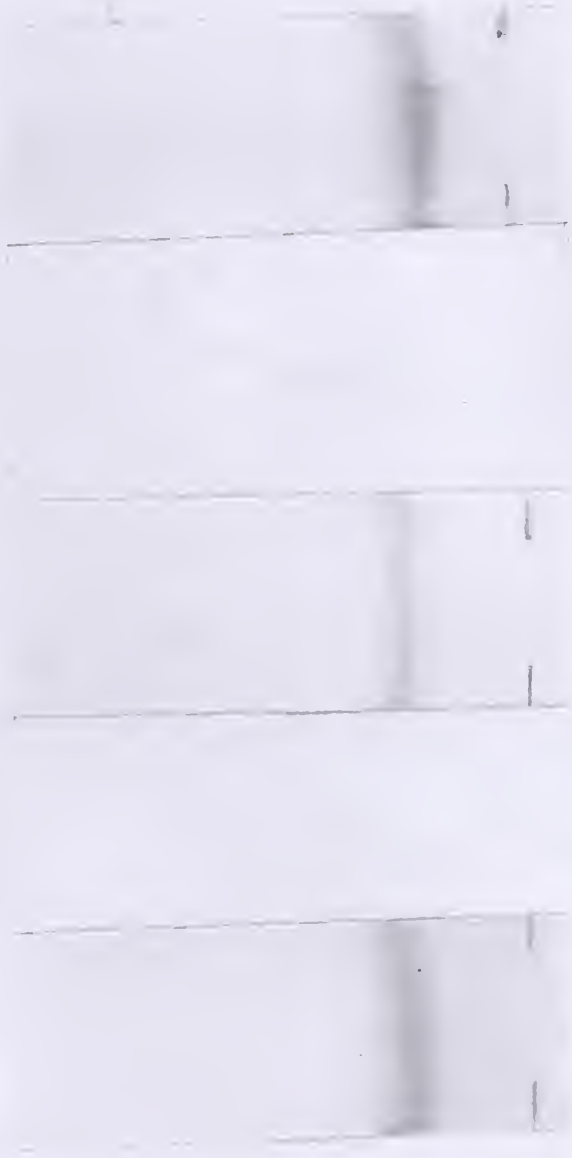




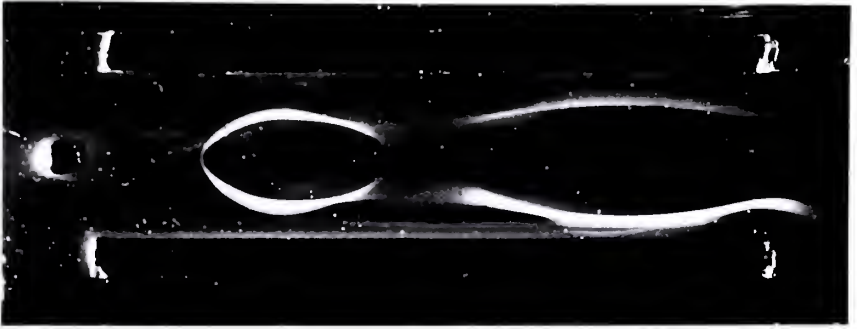
FIGURE 4.

IMMUNOELECTROPHORESIS ON AGAROSE OF PLASMA FROM A NORMAL PATIENT  
AND TWO PATIENTS F.H. AND N.T., WITH SEVERE HEPATOCELLULAR DISEASE.

The troughs contain anti-alpha and beta lipoprotein sera, R<sub>3</sub>A  
(48). Alpha lipoproteins are severely depressed in N.T. and  
F.H. All precipitation lines subsequently stained for lipid.



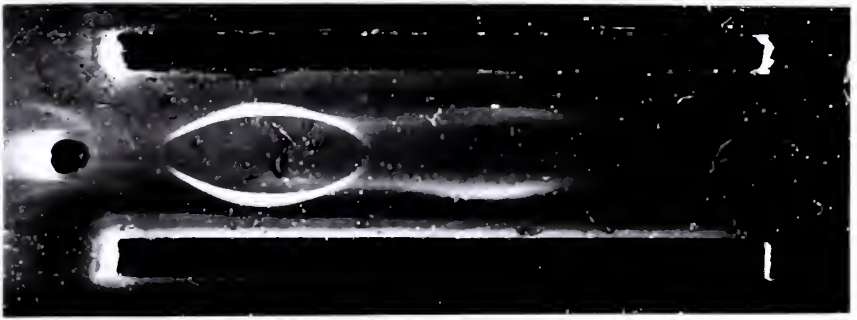
SEVERE HEPATOCYLLULAR DISEASE



Normal

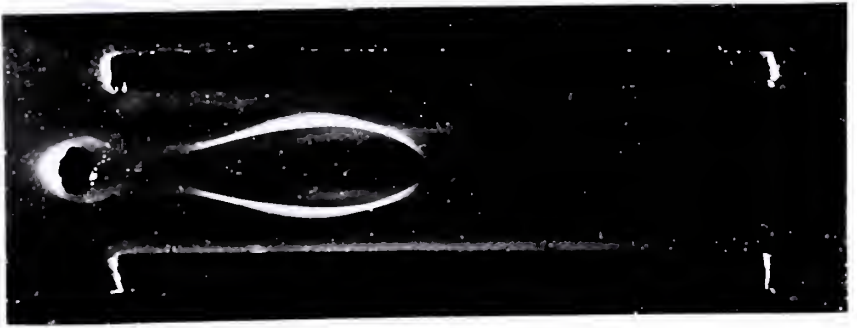
Alpha Chol.

12



N. T.

3



F. H.

1



FIGURE 5.

PAPER ELECTROPHORESIS OF PLASMA FROM PATIENT W.W. WITH BILIARY CIRRHOSIS.

The plasma, lipoprotein fraction of density  $> 1.063$  (1.063 B) and lipoprotein fraction of density  $< 1.063$  (1.063 T) have been subjected to paper electrophoresis. A broad lipid-staining band with alpha mobility is present in the plasma and fractions 1.063 T and 1.063 B.





BILIARY CIRRHOSIS

W. W. †  
11 y. o. w. ♂



PLASMA



1063 T



1063 B

CHOL. = 364 mg%  
PL = 444 mg%  
TRIG. = 92 mg%



FIGURE 6.

IMMUNOELECTROPHORESIS ON AGAROSE OF PLASMA FROM PATIENT W.C. WITH BILIARY CIRRHOSIS.

All troughs contain combined anti-alpha and beta lipoprotein sera, R<sub>3</sub>A (48).

Material with alpha lipoprotein mobility is present above and below density

1.063. All precipitation lines were subsequently stained for lipid.



W. C.  
O. Y. O., M.F.  
7/26 65



PLASMA



DENSITY < 1.063



DENSITY > 1.063



FIGURE 7.

IMMUNOELECTROPHORESIS ON AGAROSE OF PLASMA BEFORE AND AFTER  
PRECIPITATION WITH HEPARIN-MnCl<sub>2</sub> FROM PATIENT W.W. WITH  
BILIARY CIRRHOSIS.

All troughs contain combined anti-alpha and beta lipoprotein sera, R<sub>3</sub>A (48). Material with alpha mobility is present at densities above and below 1.063. All precipitation lines subsequently stained for lipid.





W. W.  
I. Y. O. W. F.

Supernatant p Heparin-AlmCl<sub>2</sub>



Plasma



Plasma



Density <math>\lt; 1.063</math>



Density >math>\gt; 1.063</math>



FIGURE 8.

IMMUNODIFFUSION ON AGAROSE OF PLASMA AND DENSITY FRACTIONS < 1.063  
(FOLLOWING PRECIPITATION) FROM PATIENT W.W. WITH BILIARY CIRRHOSIS,  
AND NORMAL ALPHA LIPOPROTEIN FRACTION.

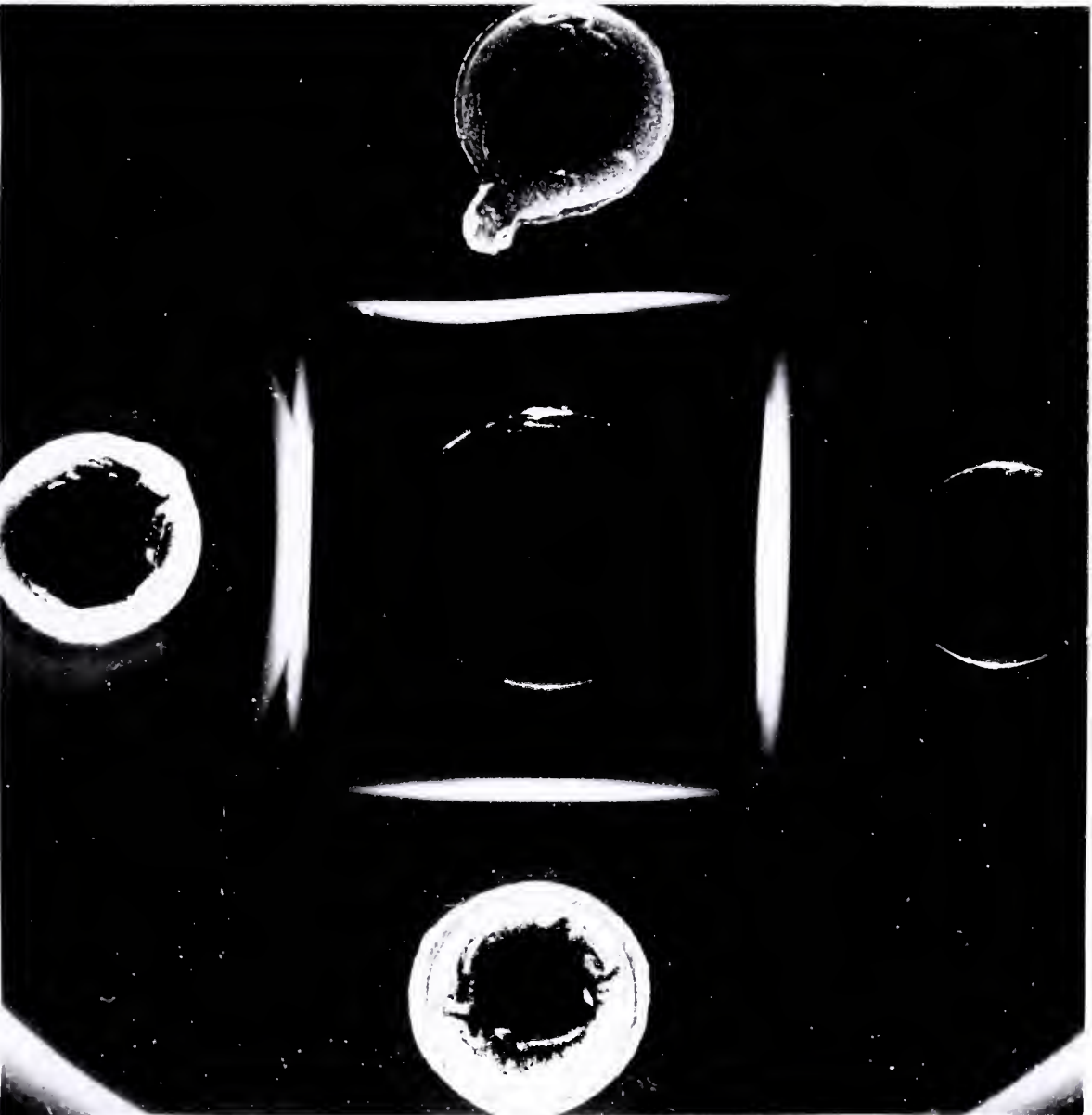
The center well contains combined anti-alpha and beta lipoprotein sera, R<sub>3</sub>A (48). The wells on the left and right contain plasma fractions 1.006-1.063 from W.W. following precipitation with either Heparin-MnCl<sub>2</sub> (Left) or specific anti-beta lipoprotein sera (Right), R7 (48). Complete identity with normal alpha lipoprotein, present in top well, was observed. The original plasma from W.W., in lower well, has both alpha and beta precipitation lines. All precipitation lines subsequently stained for lipid.



BILIARY CIRRHOSIS

NORMAL ALPHA-LP

W. W.  
11 Y.O. W.F.



DENSITY < 1.063  
p HEPARIN MgCl<sub>2</sub>

DENSITY < 1.063  
pANTI-BETA LP SERA

W.W. PLASMA



FIGURE 9.

PAPER ELECTROPHORESIS OF PLASMA FROM NORMAL PATIENT AND M. J.

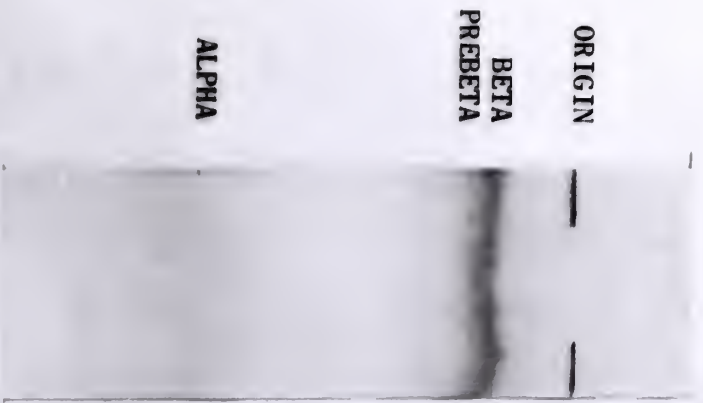
WITH BILIARY CIRRHOSIS SECONDARY TO CHOLESTATIC HEPATITIS.

On 9/15/66 with total cholesterol concentration of 2056 mgm% no lipid staining material is present in normal alpha lipoprotein position, however, an unusual cathode-migrating band is present near the origin. On 12/12/66 as total cholesterol has decreased to 668 mgm% the alpha lipoprotein band is present in normal position but in abnormally increased amount.





NORMAL



BILIARY CIRRHOSIS



ALPHA

TOTAL CHOL	198
ALPHA CHOL	<u>45</u>
BILIRUBIN	0.7
ALK. PHOS.	3.4

M.J. 9/15/66

2056

Np\*

22

235

M.J. 12/12/66

668

82\*

3

197



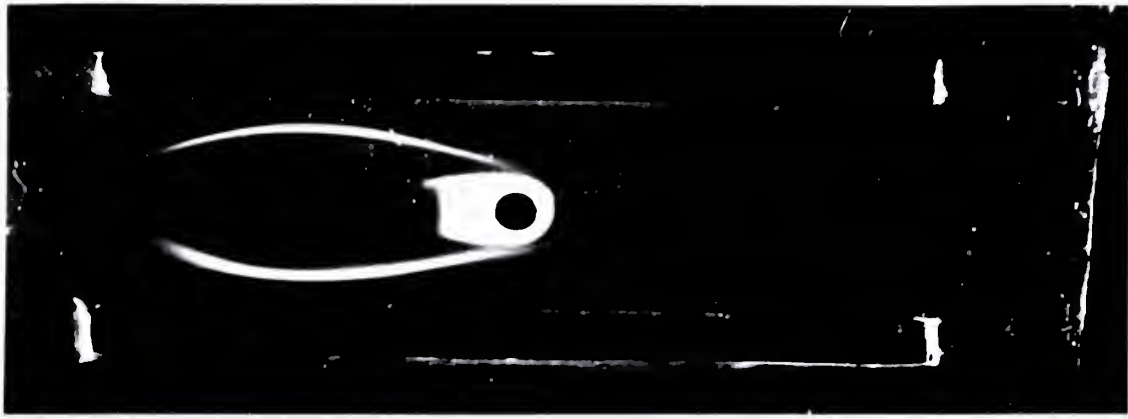
FIGURE 10.

IMMUNOELECTROPHORESIS ON AGAR OF PLASMA FROM PATIENT M.J.  
DURING ACUTE PHASE AND CONVALESCENCE FROM CHOLESTATIC  
HEPATITIS.

The troughs contain combined anti-alpha and beta lipoprotein sera, R<sub>3</sub>A (48). On 9·15 no alpha lipoprotein precipitin line is present, however, an unusual cathode-migrating precipitin line is present near the beta lipoprotein precipitin line. With return of cholesterol toward normal concentrations as seen in 12·12, alpha lipoprotein precipitin line is present in normal position. All precipitation lines subsequently stained for lipid.



M. J.  
19 y.o. N. M.



M. J.  
12/12/66



M. J.  
9/15/66



FIGURE 11.

IMMUNOELECTROPHORESIS ON AGAR OF LIPOPROTEIN FRACTION OF DENSITY 1.006-1.063 FROM M.J., 9/15/66.

Troughs in slide Density < 1.063 contain combined anti-alpha and beta lipoprotein sera R<sub>3</sub>A (48). In A antisera have been absorbed with beta lipoprotein so they now react only with alpha lipoprotein. In B antisera have been absorbed with alpha lipoprotein so they now react only with beta lipoprotein. All precipitation lines subsequently stained for lipid.





BILIARY CIRRHOSIS

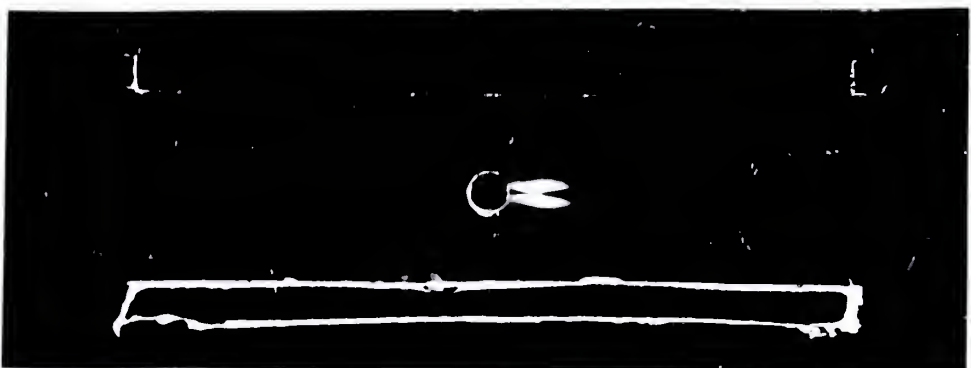
M. J. 9/15/66  
19 y.o. N. W.



DENSITY < 1.063



DENSITY < 1.063  
A



DENSITY < 1.063  
B



FIGURE 12.

IMMUNOELECTROPHORESIS ON AGAROSE OF PLASMA DENSITY 1.006 - 1.063 FRACTION FROM M.J., 9/15/66, BEFORE AND AFTER ETHER-ETHANOL DELIPIDATION.

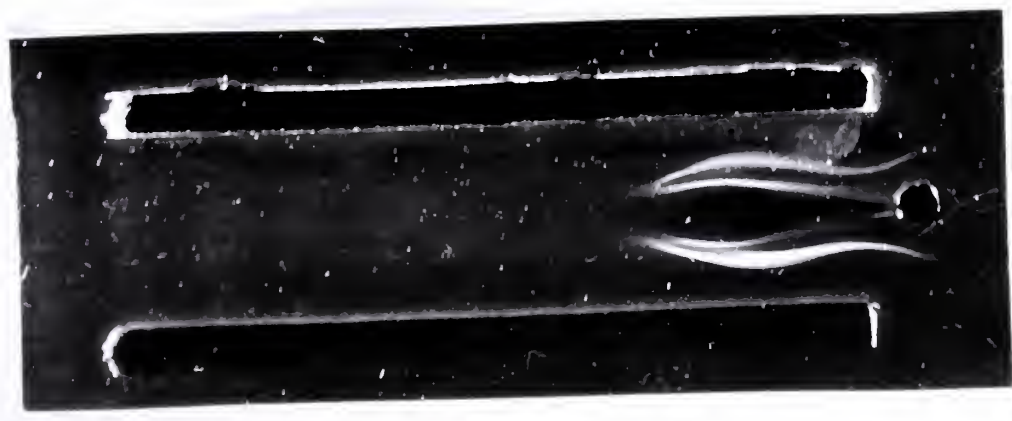
The troughs in slides Density < 1.063, and Density < 1.063 following ether-ethanol delipidation contain combined anti-alpha and beta lipoprotein sera, R<sub>3</sub>A (48). The slide Density < 1.063 following ether-ethanol delipidation A, contains specific anti-alpha lipoprotein sera R<sub>1</sub> (35), while B contains specific anti-beta lipoprotein sera, R<sub>7</sub> (48).



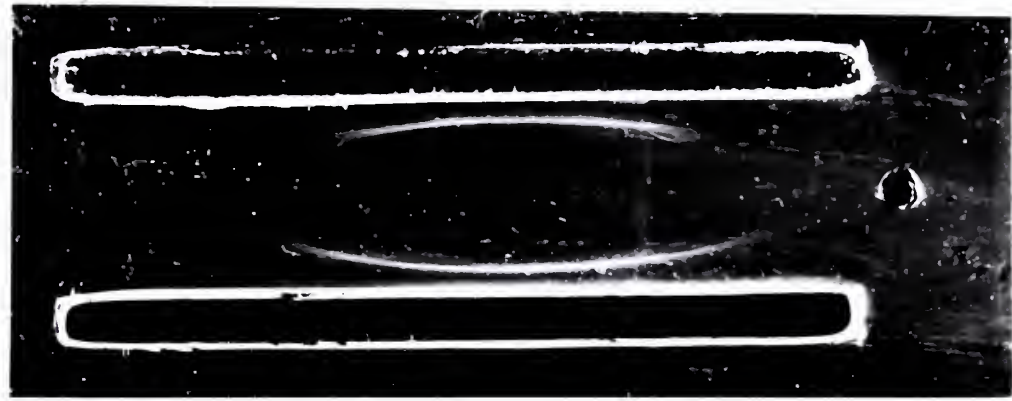
M. J.

19 y.o. N.M.

9/15/66



DENSITY  $< 1.063$



DENSITY  $< 1.063$   
 $\bar{p}$  ETHER-ETHANOL  
DELIPIDATION



A

B

DENSITY  $< 1.063$   
 $\bar{p}$  ETHER-ETHANOL  
DELIPIDATION

















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