A Clinical and Laboratory Study of Plasma Lipids in Obstructive Jaundice and Several Types of Hepatic Disease*

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CHANGES in concentration of the lipid substances present in blood, serum, or plasma which result from various types of hepatic and biliary tract disease have been extensively studied. Most observers have found that simple obstruction of the bile ducts usually produces a hypercholesterolemia (1, 2, 3, 4, 22, 25, 26, 28, 34, 38, 43, 51, 52, 70, 80, 81, 86, 87, 90, 91). This hypercholesterolemia is usually accompanied by an increase in concentration of the other blood lipids (2, 4, 28, 29, 43, 86, 90, 91). Experimental studies in animals indicate that these same changes occur after ligation of the common bile duct (23, 26, 39, 52, 80, 87). This usual finding, however, may be modified by a number of complications. The increase in concentration of the lipids in blood may be prevented from occurring, or, if already present, decreased by concomitant liver damage whether caused by chronicity of the obstruction (1, 25, 26, 87); intercurrent infection (1, 25, 26, 67); or hepatic toxins (26, 39, 52). Poor fat absorption resulting from absence of bile in the intestine in obstructive jaundice has been thought to explain absence of increased concentration of lipids by some observers (34, 78, 79).

In contrast to the effect of biliary duct obstruction, primary disease of the liver usually results in a diminished concentration of cholesterol in blood (1, 15, 25, 26, 27, 29, 40, 43, 52, 72, 81, 83, 86, 90, 91) and the other lipids behave in a similar manner (15, 27, 29, 43, 86, 90). Feigel (27) noted that ester cholesterol was diminished in concentration along with the other serum lipids in patients with acute yellow atrophy of the liver. It remained, however, for Thannhauser and Schaber (83) to correlate this reduction in concentration of ester cholesterol with impaired hepatic function. They demonstrated that the decrease in ester cholesterol paralleled the degree of hepatic damage, a finding that has been confirmed many times (1, 25, 26, 40, 43, 51, 52, 72, 81, 90). Boyd (15) has shown that the cholesterol "estersturz" of Thannhauser and Schaber is in reality a part of a general lipopenia.

The occurrence of hypercholesterolemia in patients with obstructive jaundice seems to be well established, but concerning whether this increase in cholesterol concentration results from an increase in the free or esterified cholesterol or both has led to a considerable difference of opinion. Adler and Lemmel (1), Epstein (25), Epstein and Greenspan (26), Feigel (28) and Wendt (87) find that the increase is the result of increments in both the free and ester cholesterol fractions. Epstein and Greenspan (26) find this hypercholesterolemia resulting from an increase in both the cholesterol esters and free cholesterol to be of such regular occurrence in obstructive jaundice that it assumes diagnostic significance if it can be correlated with an increased concentration of serum bilirubin. The hypercholesterolemia in obstructive jaundice has been found to result from an increase in the free cholesterol without a concomitant rise in the ester cholesterol by Boyd (14), Chanutin and Ludewig (23), Gardner and Gainsborough (34), Hawkins and Wright (39), Lehnherr (43) and Mancke (51).

Gardner and Gainsborough (34) suggest poor fat absorption as the factor which limits the concentration of ester cholesterol in the blood of patients with obstructive jaundice. Hawkins and Wright (39) could not substantiate this finding in dogs and found that decreased cholesterol esters occurred only after the liver had been injured. Chanutin and Ludewig (23) have conclusively shown, however, that in the rat, at least, the increased plasma cholesterol concentration in the presence of bile duct obstruction (ligation of the common bile duct) is due to the increased concentration of free cholesterol without a similar increase in the cholesterol esters.

In our own experience the study of cholesterol and its esters in the blood and plasma has corresponded with that of others (65, 57) (82 Snell's discussion) in that we had not obtained the useful results expected. A study of the problem (41) has shown several factors which are operative in the production of this lack of agreement with those who find the study of cholesterol and its esters and the other lipids a useful diagnostic and prognostic procedure.

A consideration of some of these factors at this point may perhaps clarify some of this confusion. Many workers have sought to compare the results of analyses of extracts of whole blood, serum and plasma. Such results, strictly speaking, are not comparable. It has been known for a long time that cholesterol is unequally distributed between the red blood cells and the plasma or serum (38, 17) and that the cholesterol present in the erythrocytes exists only as free cholesterol. This inequality of distribution of cholesterol between the cellular and fluid elements of the blood and the absence of cholesterol esters in the cells introduces an uncontrollable variable into the determination of this substance and renders impossible strict comparison of results of whole blood analyses with those of plasma or serum. Furthermore, the well known fact that oxalated plasma usually yields results lower than serum or heparinized plasma (16, 46, 77) renders strict comparison of serum and plasma questionable.

A second and most important reason for variations in results, obtained by different workers is that the data reported have been based on analyses made with

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methods which vary widely in preciseness. This applies especially to methods employed for the determination of cholesterol. Pertinent is the fact that colorimetric methods used to assay cholesterol in unsaponified extracts of blood, serum or plasma yield higher results than do the methods which isolate cholesterol before and after saponification as the digitonide, and regardless of the method subsequently employed for assay. This fact, long known (31, 44, 45, 49, 53, 54, 55, 58, 69, 88) has been shown by Gardner and Williams (35), Reinhold (69), Yasuda (92), and Kelsey (42), to result from a color enhancement effect on the Lieberman-Burchard reaction of the cholesterol esters. This enhancement effect is lost if the extract is first saponified (69). This enhancement effect produces yields of cholesterol from 20 to 30 per cent higher than the true concentration. Moreover, the variability of colorimetric methods even in skilled hands at times yields astounding errors. (See Muhlbock and Kaufmann (55), Gardner and Fox (31), Man and Peters (49)). Because of this variability of methods many reports are rendered practically useless because of poor control and by the failure of many authors to estimate the trend and magnitude of the errors inherent in the methods which they have employed.

The fact that any given value for the concentration of a substance is to be interpreted as increased or decreased by disease is predicated upon a normal range of values for that substance. A wide range of normal values renders this interpretation difficult. Such is the condition that obtains with regard to the concentration of cholesterol in the blood, serum or plasma of normal individuals. Oser and Karr (61) some time ago, and Sperry (75) more recently have pointed out that wider differences in the normal range of cholesterol concentration exist than is usually conceded. Conversely, Sperry (75) has shown that the ratio of free to total cholesterol varies within very narrow limits in health. The magnitude of these ranges will be discussed later, but it is germane at this point to indicate that the wide range of variation in cholesterol concentration in normal individuals, and the narrow limits of variation of the ratio of free to total cholesterol have contributed to the production of conflicting results.

Other factors which may contribute to the somewhat discordant results of various workers are derived from the fact that certain physiologic and pathologic factors contribute in a non-specific manner to the specific disease effect. Of these factors, age (63), sex (6, 32), race (65), obesity and normal variations in diet (5, 48) and exercise (11, 67, 68) produce little detectable effect. The effect of menstruation is usually thought to produce a decrease in concentration of cholesterol (59, 60, 56) but this is disputed by Man and Gildea (48). Body build in that the pycnic male is more apt to have serum lipid concentrations in the upper limits of normal has been found by Gildea, Kohn and Man. Lability of the vasomotor apparatus (50) and vegetative and emotional disorders indicative of basal ganglia dysfunction tend to produce increase in concentration of the serum lipids (37).

Starvation at first produced a lipemia, of more regular occurrence in the obese than in the thin individual (6), but prolongation of the starvation period induces a lipopenia associated with hypoproteinemia (47). The effect of abnormal diets is equivocal. Most workers agree that ingestion of single meal produces little changes in lipid concentration in the post absorptive state, although some workers indicate that prolonged ingestion of diets with a high fat content tends to cause an increased concentration of lipids in the blood.

Man and Gildea (48) report wide variations in the concentration of various serum lipids from time to time in individuals who have been studied for varying periods of time up to 4 years. Yet despite this wide variation of total values the variation about the mean value in this report is but slightly greater than the variations in serum cholesterol found by Turner and Steiner (85) and Sperry (76) who conclude that an individual has a mean value for total cholesterol from which large variations do not occur, and that this can be traced from month to month and year to year. Boyd (11) found little diurnal variation in the plasma lipids in young women despite exercise, meals, sleep, etc.

Among the pathologic factors which may add to the specific effect of disease, fever which produces a lipopenic effect (25), infection of any sort which causes a reduction in the ratio of free to total cholesterol (75, 82), and anemia which causes an increase in neutral fat and a decrease in phospholipid and cholesterol (65) must be mentioned. These factors as well as pathologic malnutrition are the counter parts of a host of diseases and in most cases must be active in producing some of the changes observed. Inhalation anesthetics at first produce a lipopenia (first 8 hours) and a subsequent lipemia characterized by an increase in free cholesterol (6, 12).

While the effect of many of the technical, physiological and pathological factors which may non-specifically contribute to the effect of specific disease on the concentration of the plasma lipids is known, few of the reports contain sufficient data to permit adequate evaluation of these factors. Moreover, few of the reports offer any data concerning the status of the liver with respect to its effect on the plasma lipids other than cholesterol and its esters. That study was undertaken with the idea of controlling as many of those factors as possible, and to correlate from clinical, laboratory, operative and pathological data the changes observed in the concentration of the various plasma lipids. In the interpretation of results where non-specific factors are uncontrollable, the possibility of their contribution to the effect observed can, at least, be noted.

STUDY OF THE CONCENTRATION OF PLASMA LIPIDS IN NORMAL SUBJECTS

Extracts of heparinized plasma obtained from twenty-six normal individuals were prepared by Boyd's (13) method of cold dilution, and assayed by Boyd's (14) adaptations of Bloor's (7, 8) micro oxidative procedures after slight modification (41). In our hands this method has proven quite satisfactory. With stock solutions of fatty material the average difference between duplicate determinations of fatty acids was only 3.5 per cent, although an occasional difference as great as 9 per cent was noted. With phospholipids the average difference was only 2.8 per cent with an occasional difference as great as 6 per cent. The average difference in cholesterol determi-

SUMMARY TABLE I

*Lipids**

	Total Lipid	Neutral Fat	Total Fatty Acids	Phospho-Lipids	Total	Cholesterol Ester	Free	Per Cent Free in Total Cholesterol
Range	1016-474	358-40	633-274	321-120	284-145	195-103	89-42	26.5%-32.8%
Mean	616	135	353	203	189	132	57	30.0%
S. D.	± 114	± 59	± 77.3	± 35.7	± 29.4	± 21	± 10	± 1.96
Per cent deviation	18.5	44	21.9	17.5	15.5	15.9	17.5	6.5

*Plasma proteins***

	Total	Albumin	Globulin	Fibrinogen
Range	7.88-6.41	5.58-4.18	2.74-1.86	0.61-0.32
Mean	7.19	4.91	2.28	0.44
S. D.	± 0.34	± 0.39	± 0.26	± 0.084
Per cent deviation	4.7	7.9	11.4	19.0

*Values expressed in milligrams per 100 cc. plasma.
**Values expressed in grams per 100 cc. plasma.

nations was only 1.6 per cent and in no instance was there a difference greater than 3 per cent. All digitonin used was tested against known amounts of cholesterol added to fatty solutions. Under these conditions recovery of cholesterol averaged 97 per cent which is in good agreement with Boyd's (9) finding. Despite the occurrence of several rather large errors with individual lipids, total lipids calculated from the same results never varied by more than 3.4 per cent and the average difference was only 1.4 per cent. This represents one of the advantages of the use of such a method where all lipid substances are determined or calculated from data obtained by use of the same technical procedure. Errors tend to cancel one another and one value serves as a check on the other.

Blood was collected with minimal stasis in the morning during fasting, heparinized, centrifugalized and extracts prepared and stored in glassware fitted with all glass joints. No preliminary dietary regulation was attempted, but no unusual condition was present which presumably might have altered the concentration of plasma lipids.

Plasma proteins were determined on the same sample of plasma by the method of Campbell and Hanna (19, 20, 21) to serve as a check against gross nutritional changes and hemoconcentration whether mechanically or physiologically produced.

A statistical summary of the results of these studies is presented in Table I.

For convenience in comparison of these results with those of others, Tables II and III have been compared. The differences are clearly demonstrated. Since cholesterol is the lipid substance most frequently studied, it requires more attention. Table IV has been compiled for convenience of comparison of the results of these studies with those of others. The values of cholesterol presented represent the maximum and minimum values taken from the various authors. The extremes of all these values of total cholesterol concentration in serum and plasma (109-404 milligrams per 100 cc.) demonstrate the wide range of values for this lipid in normal individuals and clearly shows how difficult is the interpretation of any given value as increased or decreased in the absence of an adequate normal control series of values regardless of what method is used.

Table V was compiled to indicate the relationship of free to total cholesterol. By inspection it may be seen that despite the wide variation in the concentration of serum or plasma cholesterol the percentage of free in total cholesterol is approximately 30 per

TABLE II

	Maximum	Minimum
Total lipoids	820	570
Neutral fat	200	0
Fatty acids	420	190
Lipoid phosphorus calculated as lecithin	330	175
Cholesterol, total	230	100
Cholesterol, free	30 to 60 per cent	
Cholesterol, esters	40 to 70 per cent	

Values are expressed in terms of Milligrams per 100 cc. of plasma.

From Peters and Van Slyke (65).

TABLE III

Author	Cholesterol Total	Cholesterol Free	Pl.	T.F.A.	N.F.	T.L.
Man and Peters* (1933)	207 ± 29	—	222 ± 29	—	6	659 ± 80
Page, Kirk, Lewis, Tompson and Van Slyke (1935)	232 ± 62	82 ± 17	181 ± 71		225 ± 137	732 ± 216
Boyd (15) (1938)	181 ± 22	53 ± 10	195 ± 37	362 ± 62	154 ± 77	617 ± 75
Present Series	189 ± 21	57 ± 10	203 ± 36	353 ± 77	135 ± 59	616 ± 114

*Estimates made by Page, Kirk, Lewis, Tompson and Van Slyke (109). Values in milligrams per 100 cc. plasma.

cent and the variation during health is within very narrow limits. The more variable results of Page, Kirk, Lewis, Tompson and Van Slyke (63) are probably explainable on the basis of an error in the method used by them as noted by Folch, Schneider and Van Slyke (30).

STUDY OF THE PLASMA LIPIDS CONCENTRATION IN SEVERAL TYPES OF HEPATIC DISEASE

The twenty-seven patients upon whom these studies were made presented a variety of forms of hepatic disease: Cirrhosis of the liver (toxic and Läennec's) (14 patients); metastatic or primary carcinoma of the liver (5 patients); hemolytic jaundice due to sulfanilamide (1 patient); toxic hepatitis due to neoarsphenamine (2 patients); atypical pigmentation of the skin associated with liver damage (2 patients); and catarrhal jaundice (2 patients). Plasma lipid and plasma protein estimations were made in the same manner as in the normal subjects but were carried out as frequently as opportunity permitted.

TABLE IV

Total cholesterol concentration in normals

Author	Maximum	Minimum
Sperry (75)	392	132
Page, Kirk, Lewis, Tompson and Van Slyke (63)	376	109
Man and Peters (49)	256	162
Pinkhardt, Bernhard and Kohn (66)	404	141
Muhlbock and Kaufmann (56)	322	147
Jones	284	145

Values in milligrams per 100 cc.

In addition during the study, various liver function tests were executed. These included biliary drainage, cholecystographic study, galactose tolerance, glucose tolerance, urobilinogen determination in urine (Wallace and Diamond), bromsulphthalein excretion (2 mg. dose of dye), hippuric acid synthesis serum phosphatase, serum bilirubin, and, at times, serum lipase determinations.

The limitations of clinical studies based on liver function tests are well known. Usually in the presence of minimal damage and occasionally in the presence of extensive damage, none of the tests may be positive. However, many of these patients were explored surgically and a more exact idea of the condition of the liver was obtained in this manner.

Plasma protein determinations were made to detect undue rapid changes in hemoconcentration and to furnish some idea of the nutritional changes in the various subjects. It is doubtful whether protein starvation with consequent production of hypoproteinemia has any effect per se on the concentration of the plasma lipids as indicated by the animal experiments of Page, Farr and Weech (62).

A summary of the results of the lipid and protein determinations from this group of patients has been made and is presented in Table VI. Comparison of the statistical summaries of the plasma lipid concentration of the normal subjects and the patients with hepatic disease shows that no statistically significant difference exists. Similar comparison of the results of the protein determinations reveals a significant decrease in plasma albumin concentration. It is interesting to note that the plasma fibrinogen concentration tends to be increased, although the increase is not great enough to be significant. The concentrations of plasma lipid are more variable in the abnormal group. Neutral fat varies most widely. This is true, also, when applied to the normal group. Neutral fat is a value calculated from residual fatty acids and hence suffers from a summation of errors in the determinations, a fact which possibly accounts for the greater variability found in this lipid. As a generalization, the wide variation of values for concentrations of the plasma lipids in both groups renders impossible the attachment of diagnostic significance to the results.

Applied to individual patients comparison of the plasma lipid concentrations of the two groups indicates that at times significant deviations are present. The number of patients in whom plasma lipid concentrations shows these significant deviations from normal is summarized in Table VII.

The most significant fact obtained from this analysis is that twenty-five of twenty-seven patients showed an increased percentage of free in total cholesterol. Each of the patients who showed that abnormality had other corroborative evidence of hepatic disease. This evidence exists in the form of positive liver function tests or abnormalities found at operation or necropsy. Roughly, the increased percentage of free in total cholesterol corresponded to the degree of hepatic damage. The group of patients who had cirrhosis of the liver usually showed the least change, although as their condition became more critical, changes in the ratio of free to total cholesterol paralleled their clinical condition regardless of whether or not jaundice was present.

TABLE V

Author	Total	Cholesterol		Per Cent Free in Total
		Ester	Free	
Gardner (33) and Gainsborough (62)	Female 153 ± 33 Male 169 ± 41	—	43 ± 17 50 ± 21	35 — 30 —
Boyd (15)	181 ± 22	128 ± 23	53 ± 10	29 —
Jones	189 ± 21	132 ± 21	57 ± 10	30 ± 2
Sperry (75)	210			27 ± 1.4
Smith and Marble (73)	215		58	28 ± 2.2
Pinkhardt, Bernhard and Kohn (66)	143-404			23 average 16-30
Muhlbock and Kaufmann (56)	147-322			30-33

In most of these patients who presented increased ratios of free to total cholesterol other factors which, presumably, may have contributed to the effect of the hepatic disease, were found. Fever and anemia were

SUMMARY TABLE VI

Plasma lipids

	Total Lipid	Neutral Fat	Total Fatty Acids	Phospho-Lipid	Cholesterol			Per Cent Free in Total
					Total	Ester	Free	
Range	1706-289	393-30	1158-151	860-88	342-72	166-5	175-37	94-25
Mean	612	170	368	223	161	89	72	47
S. D.	± 217	± 84	± 150	± 106	± 51	42	± 27	± 18
Per cent deviation	35	49	41	47	32	47	38	40

Plasma proteins

	Total	Albumin	Globulin	Fibrinogen
Range	8.80-4.21	5.52-1.92	4.70-1.76	0.82-0.18
Mean	6.70	3.52	3.17	0.49
S. D.	± 0.86	± 0.74	± 0.72	± 0.17
Per cent deviation	13	21	23	35

outstanding. The lower values for plasma total cholesterol concentration were usually associated with a diminished concentration of plasma albumin. That these changes are not the result of changes in plasma concentration is indicated by the fact that plasma albumin and globulin varied independently. These protein changes may indicate inability of the liver to synthesize plasma albumin or failing nutrition (89, 84, 74, 64, 71).

In contradistinction to the findings of Boyd and Connell (15), only three of this group of patients showed distinct and significant decreases in the concentration of all the plasma lipids. Two of these had cirrhosis of the liver (one of toxic variety, the other Läennec's type) and the other a toxic hepatitis due to neo-arsphenamine. The disease in all three terminated in death a short while after the discovery of the diminished plasma lipid concentration. Boyd and Connell state that their patients had a variety of disease conditions affecting the liver, but had no other factor which could contribute to the effect of the specific disease on the concentration of the plasma lipids. Contrariwise, in these three patients in this study, anemia, fever, infection and malnutrition were manifest and must have shared with the liver the role of production of the low level of the plasma lipid concentration. These patients all had marked diminished plasma proteins. It is suggested that nutritional factors exert a very important influence on the production of diminished plasma lipid concentration.

The nature of the plasma lipid changes found in this study are illustrated by the summaries on the studies in four patients. Case 13 was a typical example of Läennec's type of cirrhosis in whom changes in plasma lipid closely reflected changes in clinical condition of the patient. Case 22 had a toxic hepatitis due to arsphenamine and recovered. Case 23 also had

TABLE VII

Number of patients with hepatic disease showing increases in plasma lipids

	Total Lipid	Neutral Fat	Total Fatty Acids	Phospho-lipids	Cholesterol			Per Cent Free in Total Cholesterol
					Total	Ester	Free	
Beyond range in which 95% of normal values occur	2	6	2	8	1	0	14	25
Beyond range in which 66% of normal values occur	7	10	7	9	7	2	19	25

Number of patients with hepatic disease showing decreases in plasma lipids

	Total Lipid	Neutral Fat	Total Fatty Acids	Phospho-lipids	Cholesterol			Per Cent Free in Total Cholesterol
					Total	Ester	Free	
Below range in which 95% of normal values occur	3	0	3	6	11	15	1	2
Below range in which 66% of normal values occur	10	6	7	14	19	23	7	3

SUMMARY TABLE VIII

Plasma lipids (before operation)

	Total Lipid	Neutral Fat	Total Fatty Acids	Phospho-lipid	Cholesterol			Per Cent Free in Total
					Total	Ester	Free	
Range	2240-413	933-19	1406-247	939-150	511-109	249-3	275-41	99-29
Mean	987	304	610	380	238	100	138	58
S. D.	± 340	± 158	± 223	± 186	± 78	± 53	± 61	± 16
Per cent deviation	34	51	37	49	33	53	44	27

Plasma proteins (before operation)

	Total	Albumin	Globulin	Fibrinogen
Range	10.20-5.05	5.22-1.18	4.98-2.17	0.92-0.25
Mean	6.58	3.69	2.89	0.60
S. D.	± 0.80	± 0.79	± 0.56	± 0.13
Per cent deviation	12	21	19	22

a toxic hepatitis due to arsphenamine but died. Case 26 is a typical example of catarrhal jaundice with complete recovery.

STUDY OF PLASMA LIPID CONCENTRATION IN PATIENTS WITH OBSTRUCTIVE JAUNDICE

This group is comprised of nineteen patients who had varying degrees of icterus resulting from obstruction to the biliary passages. The obstruction was due to stones in twelve patients; carcinoma of the head of pancreas in three patients; carcinoma of hepatic ducts with secondary liver involvement in two patients; stricture of common duct as well as stones in one patient. In one instance the mechanism producing obstruction could not be determined.

Studies of the plasma lipids and plasma proteins and liver function tests, were executed in this group in exactly the same manner as in the other two groups.

A summary of the results of all of the plasma lipid and plasma protein determinations made before the obstruction to the outflow of bile was relieved is presented in Table VIII.

A comparison of this summary with that presented in Table I shows that this group is characterized by a statistically significant increase in free cholesterol and an increase in the ratio of free to total cholesterol. The variation which the values of plasma lipid concentrations may undergo is indicated by the percentage by which the standard deviation varies from the mean.

Comparison of this group of values (Table VIII) with the values found in the patients with hepatic diseases (Table I) shows that no difference of statistical significance is present. The wide variation in

TABLE IX

Number of patients with obstructive jaundice
Showing increased plasma lipids

	Total lipid	Neutral Fat	Total Fatty Acids	Phospho-lipids	Cholesterol			Per Cent Free in Total Cholesterol
					Total	Ester	Free	
Beyond range in which 95% of normal values occur	14	11	14	15	11	2	17	18
Beyond range in which 66% of normal values are expected to lie	16	14	17	15	14	3	18	19

Number of patients with obstructive jaundice
Showing diminished plasma lipids

	Total lipid	Neutral Fat	Total Fatty Acids	Phospho-lipids	Cholesterol			Per Cent Free in Total Cholesterol
					Total	Ester	Free	
Below range of 95% of normal values	1	1	1	1	1	1	1	0
Below range of 66% of normal values	1	1	1	1	1	1	1	0

values in the two groups renders differentiation on this basis impossible.

Deviations from the range of normal values by individual patients in this group are interesting. The nature and direction of these deviations are summarized in Table IX. Significant is the fact that free cholesterol is increased in all but two patients and cholesterol esters were increased in concentration in only three patients. An increase in total cholesterol concentration, present in only fourteen patients, indicates that the regularity of the increase in free cholesterol is more characteristic than an increase in the total. This finding corresponds with the findings of Bruger and Habs (18), Gardner and Gainsborough (34) and Boyd (14) in clinical patients, and with those of Stern and Suchantke (80) and Chanutin and Ludewig (23) in experimental animals.

An increase in the ratio of free to total cholesterol to a greater or lesser degree is present in every patient studied. This finding is usually explained on the basis of liver damage. That this group of patients had no more liver damage than would be expected in any other group of patients with obstructive jaundice is indicated by the findings at operation and the results of liver function tests. It would seem apparent that excessive liver damage need not be invoked to explain the absence of regular increase of the cholesterol esters in this material. A more likely explanation of the difference probably resides in the differences in the methods of analyses employed by some of the other workers. Moreover, gross alterations in the ratio of free to total cholesterol in the obstructed group did not necessarily indicate a fatal termination as in the patients with hepatic diseases but did reflect the seriousness of the patient's condition. Another noted difference was the fact that diminished concentrations of plasma proteins were not always associated with diminished plasma lipid concentration. Chronicity of obstruction in this material did not produce reduction in the concentration of the plasma lipids despite the fact that marked diminutions in the plasma proteins occurred.

Summaries of the studies of plasma lipid and plasma protein concentrations after release of the bile duct obstruction are presented in Table X. This summary indicates the tendency of all of the plasma lipids to return to normal. This return to normal concentration is shared by all of the lipids in a qualitative fashion. The wider variation of the free cholesterol and the ratio of free to total cholesterol is probably explainable by the fact that not all patients could be followed until complete recovery occurred.

Three patients' records are presented which are illustrative of the types of changes that occur in plasma lipid concentrations under the influence of obstruction of the bile ducts. Case 36 whose jaundice was due to a common duct stone shows those changes found in uncomplicated obstructive jaundice. This is one of the two patients who showed an increased amount of free cholesterol.

Case 34 had obstructive jaundice due to a carcinoma of the left hepatic duct which at post-mortem examination was found to have extended to involve both ducts. There was minimal hepatic involvement by the carcinoma. The long duration of the jaundice with continuous lipemia is remarkable.

Case 37 shows the characteristic effect on the plasma lipid concentration of chronic low grade obstruction to the common bile duct caused by stricture with an episode of more complete obstruction superimposed. The liver histology in this patient showed the presence of a biliary cirrhosis. It is interesting to note that this patient had a biliary fistula at the time the last plasma lipid study was made. The ratio of free cholesterol to total cholesterol was still increased even though the other liver function studies were negative. This is similar to the finding of Gardner and Gainsborough (34) who report low cholesterol esters in the presence of biliary fistulae.

SUMMARY AND CONCLUSIONS

Although the usual response of the plasma lipid concentrations to obstruction of the bile ducts and hepatic disease is in general agreed upon, many investigators arrive at discordant conclusions from their

SUMMARY TABLE X

Plasma lipids (after operation)

	Total lipid	Neutral Fat	Total Fatty Acids	Phospho-lipid	Cholesterol			Per Cent Free in Total
					Total	Ester	Free	
Range	908-413	442-76	558-224	373-100	286-94	171-15	188-38	84-29
Mean	612	190	373	203	162	85	77	49
S. D.	± 145	± 81	± 99	± 62	± 51	± 37	± 32	± 15
Per cent deviation	24	43	27	33	31	44	42	31

Plasma proteins (after operation)

	Total	Albumin	Globulin	Fibrinogen
Range	7.40-5.00	4.89-2.66	4.40-2.01	0.85-0.36
Mean	6.19	3.36	2.83	0.58
S. D.	± 0.76	± 0.54	± 0.57	± 0.12
Per cent deviation	12	16	20	20

studies. Many factors are apparent which probably have contributed to this confusion. Enumerated, these are: attempts to compare results of analyses of whole blood; serum and plasma; the use of methods of widely varying preciseness; failure to adequately control the various studies; failure to realize the wide range of normal concentration of the various lipids especially total cholesterol; failure to recognize the narrow range of variation of the normal ratio of free in total cholesterol; and failure to recognize the nonspecific contribution of various physiologic and pathologic factors to the effect on the plasma lipids. A study of plasma lipids concentration in patients with liver diseases and obstructive jaundice has been attempted with the idea of recognizing if unable to control these various factors.

1. The concentration of plasma lipids in normal individuals varies widely in states of health; especially is this true of cholesterol. But despite this wide variation in total cholesterol the ratio of free to total cholesterol varies within very narrow limits.

2. Analyses of serum or plasma with precise methods are necessary to obtain these results.

3. There was no statistically significant difference in the concentration of the plasma lipids of normal subjects and the patients with hepatic diseases studied. However, twenty-five of twenty-seven patients showed an increased percentage of free in total cholesterol and these patients had other corroborative evidence of hepatic damage.

4. Roughly the degree of change in the ratio of free to total cholesterol paralleled the seriousness of the hepatic damage.

5. A lipopenia was not found to be characteristic of the group of patients with hepatic disease. All patients showing a marked lipopenia died. Other factors such as anemia, fever and failing nutrition were present in these patients.

6. The lower concentration of total cholesterol was associated in every instance with diminished plasma albumin concentration.

7. Changes noted are not the results of variation in hemoconcentration since plasma albumin and globulin varied independently under the same conditions.

8. Patients with obstructive jaundice showed a significant increase in the concentration of free cholesterol and an increased percentage of free in total cholesterol.

9. There was no significant difference between the concentration of the plasma lipids of the group of patients with hepatic disease and those who had obstructive jaundice. Diagnosis was impossible on this basis.

10. The total cholesterol and ester cholesterol did not increase with the same degree of regularity as did the free cholesterol in the presence of obstructive jaundice.

11. Diminished plasma albumin concentration was not necessarily associated with diminished cholesterol concentration in the patients with obstructive jaundice.

12. Gross changes in the ratio of free to total cholesterol in patients with obstructive jaundice did not always indicate a fatal termination as in the patients with hepatic diseases.

13. Great care must be exercised in the interpretation of results of lipid determination since many factors, such as fever, anemia, malnutrition, etc., may play a part in producing noted changes.

14. It is suggested that malnutrition is an important but not the only factor in the production of lipopenic states in hepatic disease.

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REPORT OF CASES

Case 13. C. W. White, male, age 43. History dates from February, 1938, when the patient had an attack of jaundice associated with right lower quadrant pain. He was hospitalized at that time for one month during which the jaundice subsided. For years previous to the attack of jaundice he had been a generous consumer of alcohol. In February, 1939, he again began to have a swollen abdomen. In August, 1939, he had two abdominal paracenteses done. Stools have always been normal in color. Blood pressure 118/65. There was no visible jaundice. The liver extended 4.5 cm. below the costal margin, and the spleen was palpable. He was moderately anemic. The erythrocytes ranged between 3.28 and 3.99 million and the hemoglobin from 9.5 to 11 grams. Stools were grossly colored with bile. He was treated with a high carbohydrate diet with extra glucose feedings, bile salts, liver extract, vitamins, repeated transfusions, and diuretics. Fluid was removed from his abdomen on four occasions. Felt fairly well until 11/2/39 when he began to have nausea, and on 11/19/39 he vomited large quantities of bright red clotted blood. This bleeding was found to be associated with a prothrombin of 14 per cent. (The patient was not syphilitic). Death occurred on 11/20/39. Throughout the period of hospitalization the patient was febrile with temperature ranging from 99 to 104 degrees.

CASE 13

T.L.	N.F.	T.F.A.	Pl.	T.C.	E.C.	F.C.	% F.C.	Date	V.	B.	U.	Miscellaneous
								9- 2-39	0.5			
427	121	252	124	128	81	47	38	9- 5-39	—	30	1/500	
407	125	242	112	122	71	51	42	9-12-39				
								9-18-39			1/300	
488	188	310	123	128	83	45	35	9-23-39				
566	222	343	151	162	46	116	72	10- 6-39				
539	165	340	193	147	90	57	39	10-24-39				
519	191	325	180	125	35	90	72	11-16-39				

Case 22. A. P. Negro, male, age 25. This patient had a penile lesion at the age of 17 years. Serologic studies were positive for syphilis about three weeks before his admission on 3/31/39. He received two injections of Neo-arsphenamine in the next two weeks after finding of the positive tests for syphilis. After the first injection the patient began to have upper abdominal pain associated with nausea and vomiting but no diarrhea. He began to become jaundiced at the same time. He had one injection of the arsenical after the jaundice developed. On admission the patient was found to be deeply jaundiced but had no detectable enlargement of the liver or spleen. Feces obtained by digital examination was light in color. Duodenal intubation on 4/1/139 and again on 4/4/39 showed a free flow of bile. At the latter date the serum bilirubin was rapidly diminishing. Patient was discharged on 4/5/39 and followed in the out-patient clinic. His red cell count was 4.2 million cells, and the hemoglobin 11.5 grams. not change. During her stay in the hospital her red cell count was on two occasions over four million and the hemoglobin 11.5 grams. Throughout her stay in the hospital she was febrile. The temperature fluctuations varied between 99 and 100 degrees until the last three days of life when the temperature rose to 101 to 103 degrees. On examination there was enlargement of the liver but the spleen was not felt. Patient died 10/1/39. No autopsy was done. The clinical diagnosis was syphilis, toxic hepatitis due to arsenic.

Case 26. B. D. White, female, age 24. Admitted 4/23/39. Discharged 5/6/39 as improved. Onset of symptoms occurred 4/19/39 with upper respiratory infection. Following this, bowel irregularity developed. This latter symptom was associated with left upper quadrant pain. Dark urine was noted 4/24/39. Icterus appeared 4/25/39. On this same date some splenic enlargement was found. The

CASE 22

T.L.	N.F.	T.F.A.	Pl.	T.C.	E.C.	F.C.	% F.C.	Date	V.	B.	U.	Miscellaneous
								3-31-39				Phosphatase 3.56 units
1706	393	1158	860	342	167	175	51	4- 1-39	18.0	—	1/100	Galactose 0.197 gms.
								4- 4-39	8.5	30	1/160	
								4- 5-39				Hippuric acid 4.25 gms.
899	196	511	333	267	154	113	42	4-11-39	2.4	—	—	
								4-15-39	2.1	10	1/20	
629	113	364	256	174	119	55	32	4-22-39	0.9	8	1/20	
								4-29-39	0.4	6	1/20	
573	166	347	163	164	119	45	27	5- 6-39	0.4	4	1/20	
								5-20-39	0.3	0	1/20	
								5-27-39	0.2	4	1/20	

Temperature while in the hospital varied between 98 and 99 degrees. His therapy consisted largely of a diet high in carbohydrate reinforced with extra sugar, bile salts, limitations of fat intake. The clinical diagnosis in this case was: syphilis, toxic hepatitis due to arsenic.

Case 23. V. W. Negro, female, age 32. This patient is known to have had syphilis for several years. She had a total of 30 injections of bismuth before she was started on Neo-Arsphenamine on 7/13/39. Following the first injection of this compound she began having upper abdominal pain associated with nausea and vomiting. Several days later she noted that her eyes were icteric. Following the appearance of the jaundice she had three injections of 0.3 gram of Neo-Arsphenamine and one of 0.15 gram. The jaundice deepened during this time and the abdominal pain and nausea and vomiting became more severe until she was unable to retain anything but liquid food at the time of her admission 9/14/39. The color of the stools did jaundice subsided and patient discharged. This was typically a case of Acute Catarrhal Jaundice.

Case 34. J. M. White, female, age 50. Painless jaundice associated with clay colored stools and dark urine developed in this patient about 10/1/38. The jaundice unrelentingly became more intense until the time of her admission 11/14/38. After study she was explored 11/23/38. At operation a primary carcinoma of the left hepatic duct was found. This had extended so that there was minimal liver involvement, but the other duct was almost completely occluded. No surgical relief of the obstruction was attempted. This patient lived approximately six and one-half months after the exploration and died 5/28/39. Ascites and edema developed a short while before death. The abdomen was tapped several times for relief. Bile appeared in the stool for a short while about two months after the operation at which time there was a marked diminution in the level of the serum bilirubin. From time to time

CASE 23

T.L.	N.F.	T.F.A.	Pl.	T.C.	E.C.	F.C.	% F.C.	Date	V.	B.	U.	Miscellaneous
675	174	391	273	184	65	119	64	9-15-39	11.0	—	—	
								9-18-39	16.0	—	1/600	Phosphatase 7.4 units
								9-19-39	—	—	—	Galactose 7.32 grams
								9-22-39	12.0	—	1/400	
468	100	282	282	86	5	81	94	9-23-39				
316	33	161	180	93	15	78	84	9-26-39				
323	127	204	123	72	10	62	86	9-29-39				

CASE 26

T.L.	N.F.	T.F.A.	Pl.	T.C.	E.C.	F.C.	% F.C.	Date	V.	B.	Galactose
								4-26-39	4.3	—	—
668	259	431	198	158	79	79	50	4-28-39	6.0		4.6
								5- 1-39	4.5	—	4.0
								5- 3-39	3.4	—	—
627	141	335	224	190	107	83	44	5- 5-39	2.1		2.26
740	208	441	213	217	149	68	31	5-11-39	1.25	12	
790	218	475	246	223	155	68	30	5-18-39	0.6	—	—
								6-29-39	0.25	—	—
								8- 8-39	0.2	—	—
								10-17-39	0.2	—	—

CASE 34

T.L.	N.F.	T.F.A.	Pl.	T.C.	E.C.	F.C.	% F.C.	Date	V.	B.	U.	Miscellaneous
								11-15-38			0	Galactose 4.21 gms.
								11-17-38	8.2	—	0	
								11-19-38	17.0	—	—	
								11-28-38	15.0	—	—	
								12-23-38	27.0	—	—	
								1- 9-39	14.0	—	—	
								1-31-39	12.0	—	—	
								2- 7-39	14.0	—	—	
								2-14-39	12.0	—	—	
1283	309	792	657	255	92	163	64	2-20-39	16.0	—	—	
1437	599	951	554	282	19	263	93	3- 9-39	7.8	—	—	
1250	322	750	590	288	74	214	74	4- 6-39	12.0	—	—	
1212	377	730	512	293	45	248	85	4-24-39	16.0	—	—	
2007	933	1406	713	313	66	251	67	5- 5-39	19.0	—	—	

CASE 36

T.L.	N.F.	T.F.A.	Pl.	T.C.	E.C.	F.C.	% F.C.	Date	V.	B.	U.	Miscellaneous
								3-28-39	6.0	—	—	
								3-29-39	—	—	—	Galactose 1.8 gms.
								3-31-39	15.0	—	—	
								4- 3-39	17.5	—	—	Galactose 5.6 gms.
								4- 4-39	—	—	1/90	Urobilinogen 26.4 mg. excreted daily.
2250	642	1383	939	511	236	275	54	4- 6-39	—	—	—	
								4- 8-39	8.1	—	—	
								4-11-39	8.7	—	—	
								4-13-39	5.3	—	—	
1468	337	816	493	471	249	222	47	4-14-39	—	—	—	
								4-15-39	1.7	—	—	
								4-18-39	2.5	—	—	
								4-19-39	2.0	—	—	
738	137	404	256	237	161	76	34	4-22-39	1.7	—	—	
854	290	558	298	192	110	82	43	4-27-39	0.7	—	—	
729	209	442	250	194	114	80	41	5- 4-39	—	—	—	
								5-11-39	0.9	—	—	
634	191	392	193	168	122	46	27	5-19-39	—	—	—	

during the period after operation, this patient showed evidences of minor bleeding that was associated with marked reduction in prothrombin. By 5/6/39 she was bleeding rather profusely from most of the mucous membranes, namely, buccal mucosa, vaginal mucosa, and intestinal tract. She had no anemia at the time of her admission but late in the course of the illness a progressive hypochromic anemia appeared. Her red cell count was 3 million on 3/23/39 and 2.5 million on 5/2/39. This patient was continuously febrile for the entire post-operative period with daily temperature fluctuations that ranged between 99 and 103 degrees, except for the last two weeks of her life when her temperature was recorded as being generally below 99 degrees. A necropsy was performed.

Case 36. T. F. White, male, age 39. Admitted 3-24-39. This patient has had repeated attacks of typical biliary colic for over a year. During previous hospital admissions he has had a positive clinical diagnosis of cholecystitis and cholelithiasis made on the basis of clinical and X-ray examination. Such an attack of biliary colic occurred three days before admission following which he became slightly jaundiced. On examination at the time of admission the patient was found to be slightly overweight, and the skin and mucous membrane were deeply jaundiced. After the jaundice had subsided somewhat his abdomen was explored on 4/20/39. At this time a large stone was found in the dilated common duct. The liver was normal in size and no evidence of cirrhosis could be felt or seen. A cholecystectomy was done at that time and a T-tube was placed in the common duct. He made an uneventful recovery. At no time was the patient's blood count below normal. There was no serologic evidence of syphilis During the period before operation the patient was afebrile, and after operation fever occurred only on the first three post-operative days. He was discharged 5/15/39.

Case 37. J. C. White, female, age 64. This patient has a history of biliary tract disease dating back to 1920 when a cholecystectomy was done for the removal of numerous gall stones. During this operation the common duct was severed and an end to end anastomosis was made. She became jaundiced again a short while later and was reoperated upon for removal of small common duct stones. Since that time she has had repeated attacks of upper right quadrant pain with low grade jaundice. She has been studied in the Graduate Hospital numerous times since 1933. She was last admitted 9/15/39, following an attack of severe upper right quadrant pain and jaundice. Examination at the time of admission showed visible jaundice. Her liver was palpable about 5 cm. below the right costal border and the spleen could not be felt. After a period of study, the result of which was the conclusion that she must have residual common duct stones, her icterus became more marked. Without further delay to allow the jaundice to subside, she was explored 10/9/39. This step was taken because of the fear of more severe liver damage in this individual incident to the increased jaundice. At operation a stricture of the common bile duct was found which had a lumen about 1 mm. in diameter.

CASE 37

T.L.	N.F.	T.F.A.	Pl.	T.C.	E.C.	F.C.	% F.C.	Date	V.	B.	U.	Miscellaneous
567	166	345	157	168	113	55	33	5-28-39	1.8	25	1/20	
804	219	510	383	156	69	87	56	9-16-39				
								9-19-39	5.6	55	—	
1210	423	814	543	200	66	134	67	9-25-39	—	—	—	
								9-26-39	7.0	60	1/20	
789	153	454	363	205	102	103	51	9-28-39				
								10- 3-39	9.0	—	—	
747	319	506	267	136	38	98	73	10- 5-39	—	—	—	Galactose 0.302 gm.
814	349	556	313	136	24	112	82	10- 7-39				
704	335	470	166	162	61	101	63	10- 8-39	14.0	—	—	
658	313	456	210	116	28	88	76	10-11-39	18.0	—	—	
								10-12-39	22.0	—	—	
656	392	490	160	94	15	79	84	10-13-39	26.0	—	—	
								10-14-39	12.0	—	—	
652	442	498	100	98	18	80	82	10-15-39	7.0	—	—	
567	318	412	127	97	37	60	62	10-16-39	2.5	—	—	
468	193	309	127	107	61	46	43	10-19-39	2.6	—	—	
476	154	299	163	115	66	49	43	10-24-39	0.6	—	—	
422	114	255	149	111	71	40	36	10-28-39	—	—	—	
509	159	315	162	132	84	48	36	11- 6-39	—	—	—	
								11-17-39	0.7	—	—	
477	188	311	135	112	62	50	45	11-18-39	—	—	—	
								11-27-39	0.5	—	—	
								11-28-39	1.2	—	—	
461	165	285	131	124	61	63	51	11-29-39	—	—	—	
								12- 5-39	0.3	3	1/30	
507	144	302	168	142	79	63	44	12-14-39	—	—	—	
								12-16-39	0.2	4	0	

Proximal to the stricture there were hundreds of small pigment stones of which none was larger than the size of a pea. A plastic operation was done on the common duct and a T-tube sewn in place. As many of the small stones in the hepatic ducts were aspirated. A biopsy of the liver was made. Post-operatively there was a marked increase in the jaundice for a period of four days after which the jaundice began to clear. This patient had no evidence of syphilis or diabetes. Her red cell count was maintained between 3.8 to 4.5 million. During the post-operative period she was febrile throughout her stay in the hospital. At the time of discharge the T-tube was still in place.

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A Comparative Evaluation of the Newer Liver Function Tests*†

(Comparison of the Intravenous Hippuric Acid Test, the Cephalin-Cholesterol Flocculation Test, the Colloidal Gold Test and a Serial Bromsulphthalein Test With the Oral Hippuric Acid Test and the Rosenthal Bromsulphthalein Test)

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INTRODUCTION

IN view of the numerous functions of the liver, and the known *dissociation* of the results of different liver function tests, the importance of performing several hepatic function tests for any adequate evaluation of liver function is recognized. In this connection, it is important to determine whether the newer tests, devised during the past three years, are preferable to those in common use. Are the newer tests more sensitive than the older tests, and at the same time reliable? The purpose of this communication is to answer these questions.

A comparative statistical study has been conducted to determine the relative *sensitivity* and *reliability* of Quick's intravenous hippuric acid test, Hanger's cephalin-cholesterol flocculation test, Gray's colloidal gold test, and a modification of Macdonald's *serial* bromsulphthalein test, in relation to each other, and to the two older tests, viz., the oral hippuric acid test and the Rosenthal bromsulphthalein test.

In order to determine whether the newer tests were more sensitive, a *majority* per cent of cases with relatively *slight* or *moderate* degrees of known hepatic disease, or with suspected liver impairment, was included in the clinical material utilized. At the same time, the clinical material included also a wide variety of types and degrees of liver impairment. A sufficient number of cases with *advanced* liver disease were studied.

To evaluate the *reliability* of the newer tests, and to determine the possibility of obtaining *false* positive results in their use, it was essential to conduct careful *control* studies upon an adequate group of *normal* individuals, using each of the four newer tests.

LITERATURE

I. Rosenthal Bromsulphthalein Test

In 1924 Rosenthal and White (1) studied the physiological behavior of various chlorine, bromine and iodine phthalein dyes. These dyes, when introduced into the blood stream, were removed by the liver and excreted into the bile. Bromsulphthalein was found to be the most suitable of these dyes for use as a test of liver function. They found this dye to be non-toxic in the small doses adequate for a test of liver function.

In 1925 the same workers (2) reported the results of the clinical application of the bromsulphthalein test for hepatic function, and described their technique in detail. They advised the intravenous injection of 2 mg. of dye per kilo body weight. From the vein of the opposite arm 4 cc. of blood were withdrawn in 5

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