ULTRACENTRIFUGAL CHARACTERIZATION OF THE LIPOPROTEIN SPECTRUM IN OBSTRUCTIVE JAUNDICE: STUDIES OF SERUM LIPID RELATIONSHIPS IN INTRA- AND EXTRAHEPATIC BILIARY OBSTRUCTION [1]

By ROBERT H. FURMAN and LOYAL L. CONRAD [2] with the technical assistance of ALICE W. FRYER, BETTY S. WAMACK, and EMMA LOU McDEARMON

(From the Cardiovascular Section, Oklahoma Medical Research Foundation and the Department of Medicine, University of Oklahoma School of Medicine, Oklahoma City, Okla.)

(Submitted for publication May 28, 1956; accepted January 17, 1957)

The alterations in serum lipids accompanying obstructive jaundice have long been a matter of considerable interest. In the absence of significant hepatic failure, biliary obstruction of either intra- or extrahepatic type often results in markedly increased serum total cholesterol and phospholipid levels. The increase in serum phospholipid concentration may be relatively greater, resulting in an increased phospholipid:total cholesterol ratio. Since the increment in serum total cholesterol is due mainly to increase in the unesterified cholesterol fraction, the ratio phospholipid:free cholesterol may be more meaningful. This ratio, according to Jackson, Wilkinson, Hand, Waldron, and Vogel (1), has a minimal limiting value (by weight) of 2, suggesting a homeostatic mechanism operating to maintain a molar ratio of at least one phospholipid molecule per molecule of unesterified plasma cholesterol.

With the development of techniques such as ultracentrifugal flotation, paper and zone electrophoresis and plasma protein fractionation, the serum lipids and lipoproteins in biliary obstruction have again come under scrutiny. The serum lipid distribution in biliary obstruction has been studied by Barr, Russ, Eder, and their co-workers (2–4) employing Cohn method-10 (5) for lipoprotein fractionation. They found increased amounts of cholesterol in fractions IV + V + VI ("alpha lipoproteins"), mostly in the free form, and increased amounts of phospholipids in fractions I + III ("beta lipoproteins"). Kunkel and Slater (6), utilizing zone electrophoresis, noted marked reduction in alpha lipoprotein in biliary cirrhosis, together with increased amounts of phospholipid-rich lipid having the same relative mobility as normal beta lipoprotein. Partial ultracentrifugal characterization of the serum lipoproteins in obstructive jaundice by McGinley, Jones, and Gofman (7) indicated an increase in S_f 0–10 and 10–20 lipoproteins, usually with a rather sharp cut-off in the analytical pattern at S_f 17. The present authors extended the centrifugal characterization of the serum lipoproteins in obstructive jaundice by employing a solvent density of 1.21 gm. per L. (8). Markedly reduced or absent high density (> 1.063) lipoproteins and few, if any, low density lipoproteins of flotation rate greater than $-S_{1.21}$ 40–70 (S_f 10–20) were reported to characterize the serum lipoprotein spectrum in biliary obstruction. This centrifugal characterization was subsequently confirmed and amplified by other workers (3, 9, 10).

We wish to report additional observations in 28 patients with biliary obstruction, particularly with respect to correlations found to exist between esterified cholesterol and high density lipoprotein concentrations.

SUBJECTS AND METHODS

Patients with jaundice were selected for this study primarily from the hospitals of Oklahoma City. In most instances the etiology of the jaundice was clearly established by clinical course, biopsy or autopsy. When practicable, convalescent sera were obtained. Those subjects in whom exact diagnoses were not possible are listed as "unclassified jaundice," and the probable diagnosis is indicated.

[1] This work was supported in part by research grants from the National Heart Institute, National Institutes of Health, U. S. Public Health Service (H-1429 and H-1694), the Smith, Kline and French Foundation, and the Oklahoma State Heart Association.

[2] Dr. Conrad's work was done during the tenure of an American Heart Association Research Fellowship.

Type	No. cases
[3]Pericholangiolitic biliary cirrhosis (11, 12)	2
Biliary cirrhosis secondary to extrahepatic obstruction	2
Common duct obstruction due to a variety of lesions	6
Primary hepatoma	4
Metastatic Ca of liver, primary breast	2
Cholecystitis and cholangitis	1
Infectious hepatitis	2
Homologous serum hepatitis	3
Unclassified	6
Total	28

The van den Bergh reaction was utilized as a measure of serum bilirubin (upper normal values, total: 0.7 mg. per cent, direct: 0.4 mg. per cent) which, together with the alkaline phosphatase level (normal 2.0 to 4.5 Bodansky units per 100 ml.) provided an index of the degree of biliary obstruction. It is recognized that, in those subjects with metastatic carcinoma, some of the elevation in alkaline phosphatase levels might be due to the presence of osteoblastic activity resulting from osseous metastases. Cephalin flocculation (normal: up to 2+ in 48 hours) and thymol turbidity (normal: 0 to 4 units per 100 ml.) provided a rough index of the extent of hepatic functional impairment.

Post-absorptive serum was used for all lipid and lipoprotein determinations. Preparative ultracentrifugation was carried out according to the method of Green, Lewis, and Page (13, 14), in the Spinco Model L ultracentrifuge at 17° centigrade for 13 hours utilizing the 30.2 rotor at 30,000 r.p.m. (79,240 × gravity), solvent density 1.21 g. per ml.[4] For preparative centrifugation 2.00 grams of finely divided KBr were placed in each 10.5-ml. lusteroid centrifuge tube, 3.00 ml. of a 23.2 per cent solution of NaCl in water and 5.0 ml. of serum added.

Analytical centrifugation was accomplished in the Model E ultracentrifuge utilizing an "A" rotor at 52,640 r.p.m. (215,000 × gravity) 26.0 ± 0.5° centigrade. Analytical ultracentrifugation was sometimes repeated at a lower concentration, in order to provide $-S_{1.21}$ 25–40 (S_f 0–12) and 40–70 (S_f 12–20) lipoprotein patterns free of obscuring refractive bands. All planimetry in this study was done in triplicate by the same observer. High density $-S_{1.21}$ 0–12 lipoprotein peak areas were usually determined in the patterns obtained after centrifugation at a five-fold concentration (5 C_0) and the beta lipoprotein peak areas after centrifugation at 2 C_0 or 3 C_0. This permitted planimetry of maximum peak areas at the same time eliminating the need for extrapolation or other means of approximation. Strict attention was not paid to minor variations in flotation rates of the major lipoproteins nor was correction for Johnston-Ogston effects attempted. Conversion of area in square millimeters to milligrams per cent lipoprotein was accomplished utilizing formulation presented at the Technical Symposium of the Technical Group, Committee on Lipoproteins and Atherosclerosis, National Advisory Heart Council, 1952.

Total and unesterified cholesterol were determined essentially by the method of Sperry and Webb (16) and lipid phosphorus by a modification (17) of the method of Youngburg and Youngburg (18).

RESULTS

It was deemed necessary to provide an index of the reduction in the high density $-S_{1.21}$ 0–12 lipoprotein concentration accompanying biliary obstruction which would have meaning over a wide range of serum cholesterol values, and the values of the ratios $-S_{1.21}$ 0–12 lipoprotein:total cholesterol and $-S_{1.21}$ 0–12 lipoprotein:free cholesterol were selected for this purpose. The values of these two ratios closely approach each other as the esterified serum cholesterol fraction falls to very low levels. The values of these ratios in jaundiced subjects were then compared to those obtained from 36 and 26 sets of observations on 16 and 14 healthy male subjects, respectively, ranging in age from 20 to 72. Studies from this laboratory and from Lewis and Page (19) indicate that, in healthy subjects, the lowest $-S_{1.21}$ 0–12 lipoprotein concentrations for any given serum cholesterol concentration are seen in adult males. Since the values for the ratios $-S_{1.21}$ 0–12 lipoprotein:total cholesterol and $-S_{1.21}$ 0–12 lipoprotein:free cholesterol were not normally distributed, but evidenced marked skewing to the left, the distributions were normalized by logarithmic transformation. The mean value of the ratio $-S_{1.21}$ 0–12 lipoprotein:total cholesterol, plus or minus two standard deviations, included all but

[3] We are indebted to Dr. H. E. MacMahon, Department of Pathology, Tufts College Medical School, for review of liver biopsies and verification of the diagnosis in these two patients.

[4] It has been suggested that this method of preparative centrifugation fails to float all the cholesterol (9). Analyses of infranatant fractions obtained in the authors' laboratory after preparative centrifugation by this technique have revealed a mean of 2.6 ± 0.8 per cent of the total native serum cholesterol concentration in 72 sera from healthy subjects, and a mean of only 0.3 ± 0.3 per cent in 63 infranatant fractions in biliary obstruction (15). These observations have been extended and the adequacy and reproducibility of the removal of the top fraction by pipetting has been compared with a tube slicing method. We are satisfied that this method of preparative centrifugation is adequate and reproducible. The explanation for the higher lipoprotein values reported by Havel, Eder, and Bragdon (9) utilizing a cholesterol-concentration product is outside the parameters of this paper.

one (94 per cent), *i.e.*, the lowest of the 36 values for this ratio in the 16 healthy male subjects. The mean value less two standard deviations, namely 0.731, was then selected as the value below which the $-S_{1.21}$ 0–12 lipoprotein:total cholesterol ratio of a patient must fall in order to be considered abnormally low. The mean value of the ratio $-S_{1.21}$ 0–12 lipoprotein:free cholesterol, plus or minus two standard deviations, included all (100 per cent) of the 26 values obtained in the 14 healthy male subjects. The lowest value for this ratio observed in the normal subjects, namely 2.63, was therefore selected as the level below which the value of the ratio $-S_{1.21}$ 0–12 lipoprotein:free cholesterol in a patient must fall to be considered abnormal.

The table contains the pertinent clinical and laboratory data resulting from this study. Inspection of the data reveals that moderate to marked reduction of high density lipoprotein concentrations, evidenced by $-S_{1.21}$ 0–12 lipoprotein: total cholesterol and $-S_{1.21}$ 0–12 lipoprotein:free cholesterol ratios below 0.73 and 2.63, respectively, characterize obstructive jaundice of many different etiologies. In the two subjects with pericholangiolitic biliary cirrhosis (DP and CD) no evidence of $-S_{1.21}$ 0–12 lipoproteins was detected in more than 100 sera. The $-S_{1.21}$ 0–12 lipo-

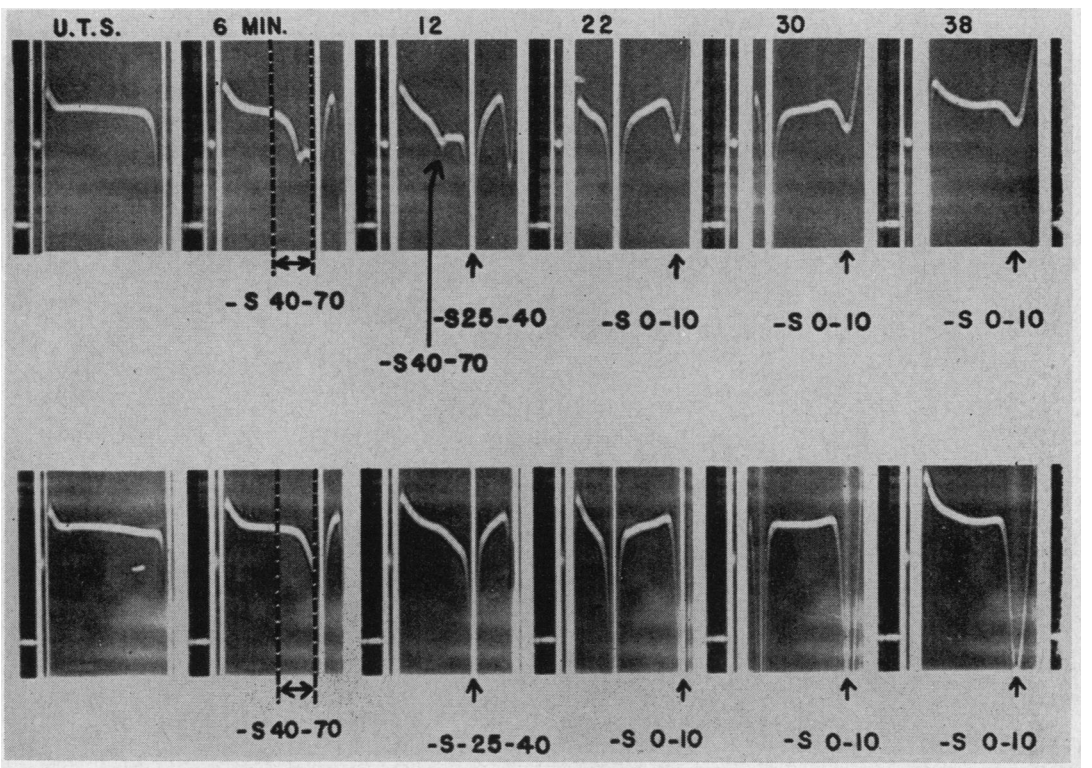

FIG. 1. PT. M.L., FEMALE, AGE 63, WITH CHOLELITHIASIS AND MINIMAL BILIARY CIRRHOSIS—REPRESENTATIVE ANALYTICAL ULTRACENTRIFUGE PATTERNS OF ICTERIC (PREOPERATIVE) AND POST-ICTERIC (POSTOPERATIVE) STAGES OF HER ILLNESS

The interval between the above analyses is approximately 3 months. Additional preoperative serum data include: total cholesterol 249 mg. per cent (esters 33 per cent), lipid phosphorus $\times 25 = 475$ mg. per cent phospholipid, van den Bergh 7 mg. per cent (total), alkaline phosphatase 11 Bodansky units per 100 ml. The post-icteric pattern was associated with the following laboratory data: total cholesterol 211 mg. per cent (esters 64 per cent), lipid phosphorus $\times 25 = 325$ mg. per cent phospholipid. In the upper (icteric) pattern the $-S_{1.21}$ 0–12 lipoprotein peak is small, there is a heavy refractive band in the $-S_{1.21}$ 25–40 (S_f 0–12) region indicating a high lipoprotein concentration and a distinct $-S_{1.21}$ 40–70 (S_f 12–20) component is visible. The lower patterns show a greater than 3-fold increase in the $-S_{1.21}$ 0–12 lipoprotein concentration, a reduced $-S_{1.21}$ 25–40 lipoprotein concentration and virtual disappearance of the $-S_{1.21}$ 40–70 class of lipoproteins. (Both analyses at 5 C_o.)

TABLE

Lipoprotein spectrum and hepatic

Date	Patient	Age	Sex	Diagnosis	van den Bergh		Alkaline phosphatase, B.U.	Thymol turbidity *units/100 cc. serum*	Cephalin flocculation (48 hours)	Total cholesterol *mg. %*	Per cent esterified	Mg. % Lipid P
					Total	Direct						
1953-54 & 55	DP	26	F	Primary (xanthomatous) biliary cirrhosis†	17.1	10.2	146	9	2–3+	550	21	50.0
1954-55	CD	55	F	Primary (xanthomatous) biliary cirrhosis‡	19.7	12.5	49	6.2	3+	514	20	45.0
2-13-54	ML	63	F	Early biliary cirrhosis, cholelithiasis	10.5	6.3	11.3		2+	249	33	19.0
5-4-54	ML				0.67	0.28	4.6			211	69	12.7
1-19-54	CAL	59	M	Biliary cirrhosis, carcinoma head of pancreas	21.6		6.4			233	18	20.4
4-5-54	CO'D	51	M	Common duct obstruction, gastric carcinoma	8.0	4.4	7.4			301	22	25.0
4-12-54	CO'D						10.0			337	19	25.1
12-8-53	OC	74	M	Common duct obstruction, carcinoma head of pancreas	16.9	10.6	14.6	1.6	1+	228	18	20.5
4-20-54	CK	74	F	Common duct obstruction, carcinoma common duct	15.0				3+	570	2	51.2
12-1-53	RW	78	F	Common duct obstruction, carcinoma head of pancreas, liver	15.3		15.6		2+	157		21.0
2-3-54	WRJ	68	M	Common duct obstruction, choledocholithiasis	2.7	1.91			Neg.	127	39	
1-19-54	CJ	64	F	Common duct obstruction, post operative adhesions	2.0	1.0	13.3		Neg.	233	56	16.0
4-6-54	CJ				2.8	1.1	14.0		Neg.	274	64	17.3
10-25-54	JW	46	M	Hepatoma, Laennec's cirrhosis	5.6	1.6	9.4	5	4+	66	42	7.9
11-29-54	JC	44	M	Hepatoma	7.68	5.44	28.2	8	2+	307		26.5
3-10-54	SW	68	F	Hepatoma	4.9	1.9			4+	100	35	6.0
1-19-55	HB	58	F	Hepatoma	3.0			6.2	4+	333	11	28.3
2-11-54	MB	47	F	Metastatic carcinoma liver, primary breast					Neg.	504	15	44.6
1-20-55	MV	51	F	Metastatic carcinoma liver, primary rectum	13.7	9.5	8.2	1.3	2+	116	11	7.6
1-19-54	NB	38	F	Cholecystitis, pericholangitis	13.9				4+	240	30	18.7
3-5-54	MS	10	M	Infectious hepatitis	4.11	4.01			3+	430	3	33.1
10-24-54	MS									134	68	9.0
7-16-55	MS				0.32					122	75	4.0
3-9-54	PM	29	M	Infectious hepatitis	3.9			15	4+	185	42	7.7
3-12-54	PM								1+	168	58	8.6
12-27-54	LW	57	M	Homologous serum jaundice	3.6	2.9	13.6	35		242	64	15.6
2-8-55	LW									198	56	14.2
3-23-54	LD	35	F	Homologous serum jaundice	17.7	14.9	4.2		4+	149	19	14.3
10-23-54	LD				0.54					153	68	9.9
4-3-54	EP	22	F	Homologous serum jaundice	5.5	2.5	2.5		4+	154	46	11.4
10-2-54	EP									225	73	11.5
7-18-55	EP									185	64	11.3
10-2-53	Van	48	M	Laennec's cirrhosis, jaundice	8.8	4.3		2		531	68	18.6
8-27-54	JA	15	M	Unclassified: probable post-hepatitis cholangiolitic cirrhosis	14.0	8.6	8.9	14.2	4+	31		4.5
8-30-54	JA									56		3.1
9-1-54	JA									26		4.2
9-3-54	JA				28.8	16.9	4.7	2.9		26		4.2
3-29-55	GN	55	M	Unclassified cirrhosis	1.1	0.6	1.9	16	4+	186	54	11.7
7-30-54	LG	54	F	Unclassified cirrhosis	14.9	6.5	6.4			134	62	9.2
8-2-54	LG				14.2	6.1	8.3		4+	113	60	9.8
8-18-54	LG									105		8.4
3-2-54	JG	77	M	Probable cirrhosis, unknown type	18.9		17.3		4+	185	29	15.6
5-19-54	GK	77	M	Undiagnosed disease, no jaundice						154	62	8.2
1-5-55	GK			Undiagnosed jaundice and hepatosplenomegaly						61	28	6.8

* "Normal" values in mg. per cent for lipoproteins determined by the method used in this study are as follows (20, 36): $-S_{1.21} > 70 = 36.7 \pm 3.8$; $-S_{1.21}$ 40–70 $= 24.2 \pm 1.8$; $-S_{1.21}$ 25–40 = 250 (approximately 20 per cent of males and 9 per cent of females have values in excess of 250 mg. per cent); $-S_{1.21}$ 20–25 $= 11.6 \pm 1.8$; $-S_{1.21}$ 1–12 $= 176.9 \pm 6.5$.

I

function in obstructive jaundice

Ratio: Mg. % Lipid P × 25 / Tot. cholesterol	Ratio: Mg. % Lipid P × 25 / Free cholesterol	Lipoproteins *mg. %** −S >70	−S 40–70	−S 25–40	−S 20–25	−S 0–12	Ratio: −$S_{1.21}$ 0–12 / Tot. cholesterol	Ratio: −$S_{1.21}$ 0–12 / Free cholesterol	Total serum Protein/Globulin *gm./100 cc.*	Remarks
2.27	2.87		664.8	704.4	Absent	Absent	0.00	0.00	7.8/3.9	Liver biopsy
2.19	2.74		766	571.1	Absent	Absent	0.00	0.00	8.9/4.0	Liver biopsy
1.91	2.84		99	Obscured	12	104	0.41	0.62	6.8/3.4	Liver biopsy
1.51	4.82		32	Obscured	12	344	1.63	5.20	7.0/3.4	2½ months postoperative, no jaundice
2.19	2.67	32	54	Obscured	5	47	0.20	0.24		Liver biopsy
2.08	2.68		77	Obscured	4.5	123	0.41	0.53		
1.86	2.30		99	Obscured	Absent	120	0.36	0.44		Laparotomy
2.25	2.74		120	Obscured	5	62	0.27	0.33	6.6/3.1	−S 40–70, triple peak. Laparotomy
2.25	2.29		29	Obscured	Absent	72	0.13	0.13		Laparotomy
3.34			30	Obscured	20	16	0.10			Laparotomy
			50	187	Absent	86	0.68	1.12	7.2/2.6	Laparotomy
1.72	3.88		32	Obscured	14	239	1.02	2.32		Laparotomy
1.58	4.46		35	Obscured	9	492	1.80	5.07		
3.00	5.21		31	151	3.6	7	0.11	0.19	6.55/3.54	Autopsy. Ceph. floc. 4+ at 24 hours
2.16			120	254	7	29	0.09		6.7/2.6	Autopsy
1.50	2.31		23	Obscured	12	9	0.09	0.14	3.7/1.6	Autopsy
2.13	2.39		86	542	Absent	72	0.22	0.24	7/?	Liver biopsy
2.21	2.60		133	Obscured	17	16	0.027	0.036		Liver biopsy, clinically jaundiced
1.64	1.84	92	81	139	Absent	158	1.36	1.53	7.0/2.9	Laparotomy
1.95	2.80		54	Obscured	3.6	43	0.18	0.25		Liver biopsy
1.93	1.98	76	94	Obscured	38	110	0.25	0.26	6.5/?	
1.68	5.36		1.8	117	14	250	1.87	5.91		Clinically well, asymptomatic
1.64	6.45		14	102	21	222	1.82	7.16		Clinically well, asymptomatic
1.04	1.79	51	53	Obscured	9	85	0.46	0.78	6.2/2.3	Cephalin 4+ in 24 hours. Clinically jaundiced
1.28	3.12	146	52	Obscured	5	84	0.50	1.22		
1.61	4.53	113	74	284	1.8	105	0.43	1.22		
1.79	4.08	61	50	320	14	279	1.41	3.23		Liver function tests all normal
2.40	2.93		94	Obscured	Absent	102	0.68	0.83	7.8/3.9	Cephalin 4+ at 24 hours
1.62	5.77		42	151	14	197	1.29	4.00		Asymptomatic
1.85	3.43	66	55	Obscured	15	90	0.59	1.08		Cephalin 4+ at 24 hours
1.28	4.64	86	52	291	12	161	0.72	2.61		van den Bergh normal 5-7-54
1.53	4.29		105	198	12	165	0.89	2.48		Asymptomatic
0.88	2.77	93	72	Obscured	8.5	79	0.15	0.48	5.4/3.0	Still jaundiced 2 years later
3.65			9	102	1.8	5	0.18		8.7/6.1	Liver biopsy
1.39			14	87	1.8	11	0.20			
4.04			13	90	5	9	0.36			
4.04			16	86	4.5	16	0.63		7.1/4.8	
1.58	3.41	72	58	267	3.6	182	0.98	2.11	7.4/4.6	Liver biopsy
1.72	4.51		36	159	4.5	169	1.27	3.31	8.0/4.3	
2.17	5.33		25	157	8	142	1.25	3.10		
2.00			23	161	10	64	0.60			
2.05	2.88		80	Obscured	Absent	41	0.23	0.32	6.5/3.2	Liver biopsy not diagnostic
1.33	3.48		32	Obscured	3.6	121	0.78	2.03		
2.79	3.86		41	128	Absent	73	1.21	1.67		Deeply jaundiced, deceased 1-7-55

† Values reported are means of serial observations made over 2-year period.
‡ Values reported are means of serial observations made over 6-month period.

protein:total cholesterol ratio was determined in all 28 patients and in 23 it fell below 0.73. One of the five patients whose ratios exceeded this value had a value of only 0.78 (GK). The $-S_{1.21}$ 0–12 lipoprotein:free cholesterol ratio, determined in 25 of these patients, fell below 2.63 in 24 cases. One or both ratios were abnormally low in 27 of the 28 patients in the study. As a rule the lower values of these ratios were seen in the more deeply jaundiced patients, but there were exceptions (*e.g.*, LG).

When jaundice subsides following relief of obstruction, high density $-S_{1.21}$ 0–12 lipoprotein levels increase (Figure 1), serum free cholesterol levels return toward normal (if previously abnormal) resulting in an increase in the per cent of cholesterol esterified, and the ratios $-S_{1.21}$ 0–12 lipoprotein:total cholesterol and $-S_{1.21}$ 0–12 lipoprotein:free cholesterol increase to or above values for the normal adult male. As improvement continues, elevated concentrations of low density $-S_{1.21}$ 25–40 and 40–70 lipoproteins fall toward normal, resulting in increased $-S_{1.21}$ (0–12/25–70) lipoprotein ratios. If progressive disease is present, further decrease in the value of these ratios is usually noted.

The data indicate that most of the serum cholesterol and phospholipids, even when markedly increased in amount, are present as low density $-S_{1.21}$ 25–40 (S_f 0–12) lipoproteins, and to a lesser degree, as lower density lipoproteins of $-S_{1.21}$ 40–70 (S_f 12–20) class. Inhomogeneity of the low density group of lipoproteins was occasionally evidenced by the appearance of multiple peaks in the $-S_{1.21}$ 40–70 region of the analytical patterns (Figure 2).

The presence of low density lipoproteins of flotation rates in excess of $-S_{1.21} > 70$ ($S_f > 20$) was noted in only eight subjects, and no correlation was apparent between these low density lipoproteins and other laboratory or clinical data, with the possible exception of hepatitis, since five of six subjects with either homologous serum jaundice or infectious hepatitis exhibited relatively small amounts of these lipoproteins.

It is of interest to note that the value of the ratio phospholipid:free cholesterol was less than 2.0 in only three subjects (MV, metastatic carcinoma, 1.84; MS, and PM, each with infectious hepatitis, 1.98 and 1.79, respectively). This study amply confirms the observations of Jackson and his co-workers (1) mentioned previously. In this regard it is of further interest to note that phospholipid:free cholesterol ratios in native serum as well as in Cohn fractions IV + V + VI, and I + III in a healthy woman and in a woman with primary biliary cirrhosis, calculated from the data of Table I in the paper by Russ, Raymunt, and Barr (10), do not exceed the value 2.0 in any instance.

There is a positive correlation between the serum albumin and total cholesterol levels ($r = 0.585$, significant at the 0.02 level) in the 15 subjects in whom a total of 17 albumin levels was determined.

DISCUSSION

The mechanism or mechanisms responsible for the increment in serum free cholesterol and phos-

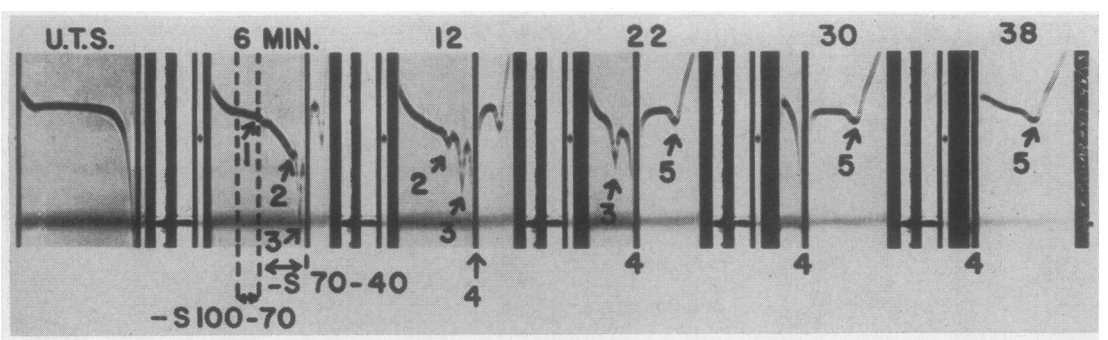

FIG. 2. PATIENT O.C., MALE, AGE 74, WITH COMMON DUCT OBSTRUCTION DUE TO CARCINOMA OF THE HEAD OF THE PANCREAS—ANALYTICAL ULTRACENTRIFUGE PATTERN AT 5 C_0 SHOWING THREE "SUB-SPECIES" OF LOW DENSITY LIPOPROTEINS UNDERGOING FLOTATION WITHIN THE LIMITS $-S_{1.21}$ 40–70 (No.'s 1, 2, AND 3)

The principal lipoprotein peak (No. 4) is obscured by a refractive band due to the high concentration present. The No. 5 peak is a high density lipoprotein here undergoing flotation at an average rate of approximately $-S_{1.21}$ 12.

pholipid levels, and for the failure of the esterified cholesterol fraction to increase, are not understood. Simple retention of bile, increased plasma cholate (20), alteration in serum cholesterol esterifying enzyme activity (21, 22) and increased hepatic cholesterol synthesis (23) have been suggested in explanation. While as much as 50 per cent of the cholesterol absorbed from the intestinal tract is present as cholesterol ester in the thoracic duct lymph (24), animal studies by Friedman and Byers (25) indicate that the liver is importantly concerned with the supply as well as withdrawal of plasma ester cholesterol. Inability of the liver to remove cholesterol from the blood for esterification may be an important reason for the relatively or absolutely low levels of plasma ester cholesterol commonly seen in biliary obstruction. Inspection of the table indicates that in only one instance (Van) out of 37 determinations did the absolute level of esterified cholesterol exceed the upper range of normal for this fraction (200 mg. per cent), while 15 determinations were below the lower range of normal (70 mg. per cent). The remaining values were within commonly accepted normal limits by the methods employed for their determinations.

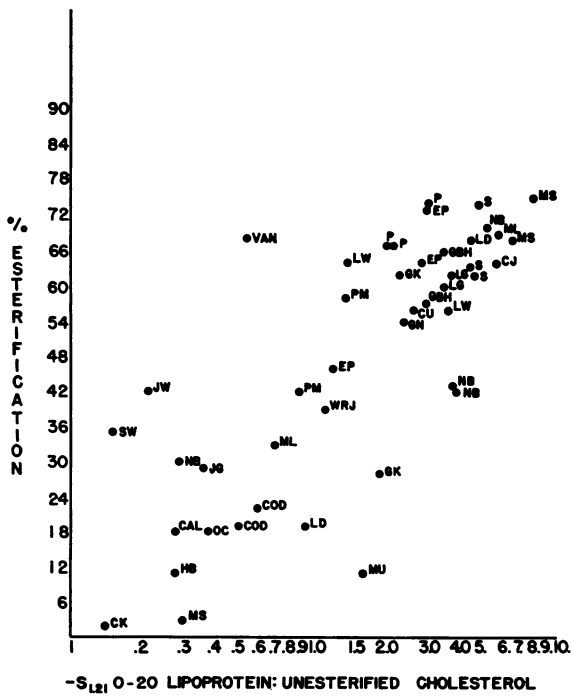

FIG. 3. SEMILOGARITHMIC PLOT OF PER CENT CHOLESTEROL ESTER (ORDINATE) AS A FUNCTION OF THE RATIO, $-S_{1.21}$ 0–20 LIPOPROTEIN : UNESTERIFIED CHOLESTEROL (ABSCISSA), IN 26 SUBJECTS DURING ICTERIC AND POST-ICTERIC PHASES OF HEPATOBILIARY DISEASE

The coefficient of correlation (r) between these two functions is 0.77 ($p < 0.001$), z (35) value 1.02 ± 0.155. The regression equations are: $y = 33.9 \times \pm 41.8$ and $x = 0.018\ y - 0.683$.

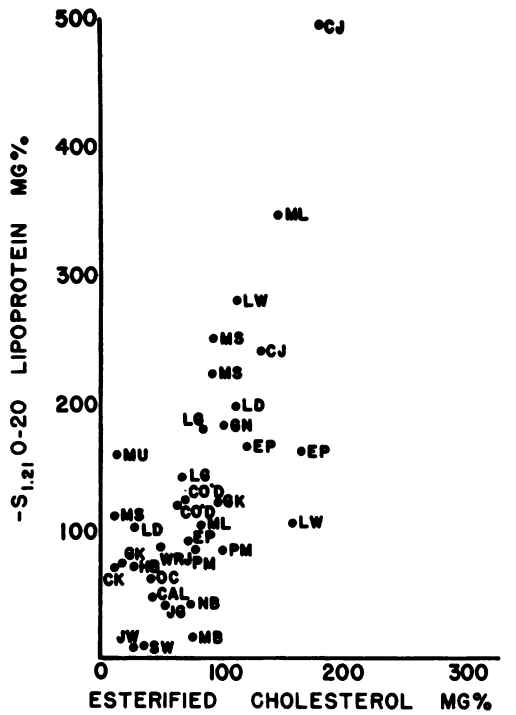

FIG. 4. RECTILINEAR PLOT OF ESTERIFIED CHOLESTEROL CONCENTRATION (ABSCISSA) AS A FUNCTION OF THE $-S_{1.21}$ 0–20 LIPOPROTEIN CONCENTRATION (ORDINATE)

The coefficient of correlation (r) between these two functions is 0.68, ($p < 0.001$) z value 0.82 ± 0.18. The regression equations are $y = 0.3x + 36.6$ and $x = 1.5\ y + 16.8$.

Because reduced $-S_{1.21}$ 0–12 lipoprotein concentrations and $-S_{1.21}$ 0–12 lipoprotein:free cholesterol ratios, as well as lower esterified cholesterol fractions, are common to obstructive jaundice, these data were examined for possible correlations. In Figure 3 the value of the ratio $-S_{1.21}$ 0–12 lipoprotein:free cholesterol is plotted semilogarithmically as a function of the per cent esterification of the serum cholesterol in 26 patients in whom these data are available from 45 determinations. In Figure 4 the concentration of esterified cholesterol is plotted on rectilinear coordinates as a function of the $-S_{1.21}$ 0–12 lipoprotein concentration in these same subjects. Both plots show a highly significant positive correlation. It is possible therefore to predict the $-S_{1.21}$ 0–12 lipoprotein concentration in patients

with obstructive jaundice from knowledge of either the concentration of the cholesterol esters, or the total or free cholesterol concentration, together with the per cent of cholesterol esterified.

The metabolic significance of these correlations is not evident from this study. Several possibilities suggest themselves. The factors responsible for the relative inability of the liver to esterify cholesterol in obstructive jaundice, or for the reduction in cholesterol-esterifying enzyme activity, may retard or otherwise inhibit the synthesis of high density lipoproteins. Alternatively, these factors may modify the lipid composition of these lipoproteins, reducing their density.

From the serum of healthy subjects Cohn fractions IV + V + VI yield lipoproteins which appear to be similar to if not identical with the high density $-S_{1.21}$ 0–12 lipoproteins. It is clear that this similarity no longer exists in obstructive jaundice, since in this disorder the lipid recovery from Cohn fractions IV + V + VI is increased (2, 3, 9, 10). It is possible that high density $-S_{1.21}$ 0–12 lipoproteins acquire additional lipid in obstructive jaundice, thereby rendering them "low density" lipoproteins, but retain solubility characteristics that determine their presence in the Cohn fractions IV + V + VI. Other differences have been suggested by Russ, Raymunt, and Barr (10). Reported analyses of the high density (> 1.063) lipoproteins found in the serum of patients with biliary obstruction are scant (9, 10) and do not take cognizance of the relatively large "lipid" phosphorus fraction (5 to 10 per cent of the "lipid" phosphorus concentration of native serum) found in the 1.063 and 1.21 "infranates" which is insoluble in petroleum ether (9 addendum, 15). Further studies of these lipoproteins are necessary.

The observations we have been able to make in two patients with metastatic carcinoma of the liver (MB and MV) are of interest in view of the reports by other workers (26, 27) in which low "alpha" lipoprotein levels were noted in advanced carcinoma of the breast, especially when bone metastases were present. It is possible that the reduced "alpha" lipoprotein concentrations in these patients are due in significant measure to liver metastases which lead to some degree of biliary obstruction. The lowest values for the ratios $-S_{1.21}$ 0–12 lipoprotein:total cholesterol and $-S_{1.21}$ 0–12 lipoprotein:free cholesterol in the studies reported here were noted in the subject with carcinoma of the breast with metastases (MB). High density $-S_{1.21}$ 0–12 lipoproteins are readily diminished by androgenic and increased by estrogenic steroids (28). Studies of the serum lipoproteins in patients with breast cancer, therefore, must take cognizance of any recent therapy with gonadal steroids or related compounds.

It is likely that a variety of types of liver damage may reduce the ability of the liver to synthesize high density lipoproteins. Total body radiation studies described by Entenman, Neve, and Olmstead (29) and by others (30) suggest the possibility that liver damage may result from such radiation and be reflected in the plasma by a reduced level of high density lipoproteins. In view of the increase in high density lipoprotein concentration caused by estrogen administration, it is interesting to speculate on the mechanism of the protection against acute radiation mortality that estrogens afford (31–34).

SUMMARY AND CONCLUSIONS

Serum lipid and lipoprotein determinations were made in 26 subjects with obstructive jaundice of varying etiology. Ultracentrifugation employing a solvent density of 1.21 g. per ml. was utilized for lipoprotein analysis.

The following conclusions were derived:

1. Obstructive jaundice, regardless of type, is characterized by: (a) reduced or absent high-density (> 1.063) lipoproteins of flotation rate $-S_{1.21}$ 0–20; (b) increased $-S_{1.21}$ 25–40 and $-S_{1.21}$ 40–70 lipoproteins.

2. Low density lipoproteins of the flotation rate class $-S_{1.21}$ 70–100 may be present in the serum from patients with homologous serum hepatitis, but as a rule are absent in other types of obstructive jaundice.

3. There exists a highly significant and positive correlation between the per cent esterification of serum cholesterol and the logarithm of the ratio $-S_{1.21}$ 0–20 lipoprotein:free cholesterol. (This ratio was selected to provide an index of high density lipoprotein levels in relation to the serum cholesterol concentrations.)

4. There exists a highly significant and positive correlation between the concentrations of esterified cholesterol and $-S_{1.21}$ 0–20 lipoproteins.

5. The concentration of high density lipoproteins in obstructive jaundice may be predicted from knowledge of either the concentration of the cholesterol esters or the total or unesterified cholesterol concentration together with the per cent of cholesterol esterified.

ACKNOWLEDGMENTS

The authors wish to express their sincere thanks and appreciation to the many staff members of Wesley, Mercy, St. Anthony, Veterans and University Hospitals, and to the several physicians of the State whose help and cooperation made this study possible.

Dr. Carl R. Doering and Mr. F. W. Ashley of the Department of Preventive Medicine and Public Health, University of Oklahoma School of Medicine, have rendered invaluable assistance in the statistical analysis of the data presented. The authors wish to express their gratitude for this important contribution.

REFERENCES

1. Jackson, R. S., Wilkinson, C. F., Jr., Hand, E. A., Waldron, A. M., and Vogel, W. C., The relationship between the phospholipids and the cholesterols in human plasma and lymph. Proc. Rudolf Virchow Medical Society, New York, 1952, **11**, 99.
2. Barr, D. P., Russ, E. M., and Eder, H. A., Protein-lipid relationships in human plasma. II. In atherosclerosis and related conditions. Am. J. Med., 1951, **11**, 480.
3. Eder, H. A., Russ, E. M., Pritchett, R. A. R., 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.
4. Eder, H. A., and Russ, E. M., Plasma protein-lipid relationships in acute hepatitis. J. Clin. Invest., 1953, **32**, 564.
5. Cohn, E. J., Gurd, F. R. N., Surgenor, D. M., Barnes, B. A., Brown, R. K., Derouaux, G., Gillespie, J. M., Kahnt, F. W., Lever, W. F., Liu, C. H., Mittelman, R. F., 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.
6. Kunkel, H. G., and Slater, R. J., Lipoprotein patterns of serum obtained by zone electrophoresis. J. Clin. Invest., 1952, **31**, 677.
7. McGinley, J., Jones, H., and Gofman, J., Lipoproteins and xanthomatous diseases. J. Invest. Dermat., 1952, **19**, 71.
8. Furman, R. H., Conrad, L. L., and Howard, R. P., A serum lipoprotein pattern characteristic of biliary obstruction, with some comments on "jaundice due to methyltestosterone." Circulation, 1954, **10**, 586.
9. 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.
10. Russ, E. M., Raymunt, J., and Barr, D. P., Lipoproteins in primary biliary cirrhosis. J. Clin. Invest., 1956, **35**, 133.
11. MacMahon, H. E., Biliary xanthomatosis (xanthomatous biliary cirrhosis). Am. J. Path., 1948, **24**, 527.
12. MacMahon, H. E., Biliary cirrhosis, differential features of the five types. Lab. Invest., 1955, **4**, 243.
13. Green, A. A., Lewis, L. A., and Page, I. H., A method for the ultracentrifugal analysis of α and β serum lipoproteins. Federation Proc., 1951, **10**, 191.
14. Lewis, L. A., Green, A. A., and Page, I. H., Ultracentrifuge lipoprotein pattern of serum of normal, hypertensive and hypothyroid animals. Am. J. Physiol., 1952, **171**, 391.
15. Furman, R. H., Norcia, L. N., Fryer, A. W., and Wamack, B. S., Lipoprotein recovery following ultracentrifugal fractionation at solvent density 1.21 as determined by cholesterol and lipid phosphorus analyses of supernatant and bottom fractions. J. Lab. & Clin. Med., 1956, **47**, 730.
16. Sperry, W. M., and Webb, M., A revision of the Schoenheimer-Sperry method for cholesterol determination. J. Biol. Chem., 1950, **187**, 97.
17. Hawk, P. B., Oser, B. L., and Summerson, W. H., Practical Physiological Chemistry, 12th ed., Philadelphia, Blakiston Co., 1947, p. 541.
18. Youngburg, G. E., and Youngburg, M. V., Phosphorus metabolism. I. A system of blood phosphorus analysis. J. Lab. & Clin. Med., 1930, **16**, 158.
19. Lewis, L. A., and Page, I. H., Electrophoretic and ultracentrifugal analysis of serum lipoproteins of normal, nephrotic and hypertensive persons. Circulation, 1953, **7**, 707.
20. Friedman, M., and Byers, S., Production and excretion of cholesterol in mammals. VI. Bile acid accumulation in production of hypercholesteremia occurring after biliary obstruction. Am. J. Physiol., 1952, **168**, 292.
21. Sperry, W. M., Cholesterol esterase in blood. J. Biol. Chem., 1935, **111**, 467.
22. Turner, K. B., McCormack, G. H., Jr., and Richards, A., The cholesterol-esterifying enzyme of human serum. I. In liver disease. J. Clin. Invest., 1953, **32**, 801.
23. 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. Exper. Med., 1954, **99**, 43.

24. Chaikoff, I. L., Bloom, B., Siperstein, M. D., Kiyasu, J. Y., Reinhardt, W. O., Dauben, W. G., and Eastham, J. F., C-14 cholesterol. I. Lymphatic transport of absorbed cholesterol-4-C-14. J. Biol. Chem., 1952, **194**, 407.
25. Friedman, M., and Byers, S. O., Observations concerning the production and excretion of cholesterol in mammals. XVI. The relationship of the liver to the content and control of plasma cholesterol ester. J. Clin. Invest., 1955, **34**, 1369.
26. Barclay, M., Cogin, G. E., Escher, G. C., Kaufman, R. J., Kidder, E. D., and Petermann, M. L., Human plasma lipoproteins. I. In normal women and in women with advanced carcinoma of the breast. Cancer, 1955, **8**, 253.
27. Kaufman, R. J., Barclay, M., Kidder, E. D., Escher, G. C., and Petermann, M. L., Human plasma lipoproteins. II. The effect of osseous metastases in patients with advanced carcinoma of the breast. Cancer, 1955, **8**, 888.
28. Furman, R. H., Atherosclerosis and lipoproteins. South. M. J., 1955, **48**, 6.
29. Entenman, C., Neve, R. A., and Olmstead, C. A., Species differences in plasma phospholipid levels as influenced by whole-body x-irradiation. Federation Proc., 1953, **12**, 40.
30. Milch, L. J., Yarnell, R. A., Stinson, J. V., and the Cardiovascular Research Group, Relationship between blood lipids and radiation injury in rabbits. Science, 1954, **120**, 713.
31. Mirand, E. A., and Hoffman, J. G., Protective action of estrogens given after radiation dose. Radiation Res., 1954, **1**, 554.
32. Mirand, E. A., and Lasser, E. C., Hormonal modification of tissue reactions to ionizing radiation. Exp. Med. & Surg., 1955, **13**, 234.
33. Rugh, R., and Clugston, H., Radiosensitivity with respect to the estrous cycle in the mouse. Radiation Res., 1955, **2**, 227.
34. Rugh, R., and Wolff, J., Relation of gonad hormones to x-irradiation sensitivity in mice. Proc. Soc. Exper. Biol. & Med., 1956, **92**, 408.
35. Fisher, R. A., On the "probable error" of a coefficient of correlation deduced from a small sample. Metron, 1921, **1**, 3.
36. Lewis, L. A., The lipoprotein system. Minnesota Med., 1955, **38**, 775.