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LIPOPROTEIN METABOLISM AND LIVER DAMAGE

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(Thesis)

July 22, 1953

Berkeley, California

Table of Contents

LIPOPROTEIN METABOLISM AND LIVER DAMAGE

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July 22, 1953

Abstract

Normal and cholesterol fed rabbits were injected with carbon tetrachloride and their serum cholesterol levels and lipoproteins of the S_f 3-12, 12-20, and 20-40 classes measured during and after the injections. Carbon tetrachloride produced a marked increase above control levels in all classes of lipoproteins and in cholesterol in the non-cholesterol fed rabbit. These substances gradually decreased to control levels after the cessation of the carbon tetrachloride injections. In the cholesterol fed rabbit, cholesterol and all classes of lipoproteins increased during carbon tetrachloride injections but continued to increase after the cessation of carbon tetrachloride injections.

The serum levels of the S_f 10-20 class of lipoproteins measured in 32 patients with chronic hepatitis were at least as high and possibly higher than those found in clinically normal individuals of corresponding age and sex.

The S_f 0-12, 12-20, and 20-100 classes of lipoproteins are significantly elevated in patients with infectious or serum hepatitis when compared with normals of matched age and sex. The S_f 100-400 class of lipoproteins is significantly reduced. The flotation rate of the major peak in the S_f 0-12 class is significantly increased in these patients. Lipoprotein measurements, however, do not segregate infectious from serum hepatitis.

There is a significant positive correlation in acute hepatitis between the S_f 0-12, 12-20, 20-100 classes of lipoproteins or the flotation rate of the major peak in the S_f 0-12 class and the icterus index or serum bilirubin (either direct or total). There is a significant negative correlation between the S_f 100-400 class and the icterus index or serum bilirubin.

The entire S_f 0-400 group of lipoproteins correlates significantly with the thymol turbidity test although no subclass within this group correlates significantly. The S_f 20-100 class of lipoproteins correlates significantly with the total lipid determination of Kunkel, Ahren, and Eisenmenger (phenolic precipitation). The return of lipoprotein levels to normal during convalescence, however, parallels the return of the icterus index to normal.

Normal rabbits were injected with lipoproteins isolated from cholesterol fed rabbits. Lipoproteins of the S_f 5-15, 15-20, 20-100, 100-400, and 400 + classes were studied. Within a few hours, lipoproteins of high S_f rate converted to lipoproteins of lower S_f rate in a serial fashion. Conversion was always from high to low and never in the reverse direction. Conversion of lipoproteins of high S_f rate to lower S_f rate was accompanied by a progressive lowering of concentration in the lower S_f classes. Eg: S_f 100-400 → less S_f 30-100 → still less S_f 15-30, etc.

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The lipids in serum are carried in the form of giant molecules which are at least as large as the albumin molecule although many are much larger. Cohn, Oncley, and collaborators were able to separate two classes of lipid-containing molecules using salt-ethanol fractionation of serum. They named these two classes the α and β lipoproteins, and were able to account for much of the serum lipids in these two lipoprotein groups. This work is excellently summarized in a review article by Edsall[1]. Gofman[2] used the analytical ultracentrifuge in studying the serum lipoproteins, and found that each of the two classes of Cohn and Oncley were actually composed of many different lipoprotein species. The α lipoprotein class contained at least three species, and the β lipoprotein class was composed of a great number of species, numbering perhaps in the thousands.

By increasing the density of serum, a critical point may be reached where all the lipoproteins float to the top and the proteins sediment to the bottom in an appropriate ultracentrifugal field. If the top lipoprotein containing fraction is analyzed for lipids, it is found that over 95 percent of the cholesterol, phospholipid, and neutral fat in the original serum are found in the top fraction. In addition, protein is also present; hence the name lipoprotein as applied to the giant lipid containing molecules. Lindgren et al[3] have studied isolated lipoproteins. They found that in all species studied some protein is found, although in varying percentage. If sufficient salt is added to raise the serum density to 1.2, all lipoproteins can be made to float to the top of the ultracentrifuge tube if centrifugation is performed for 24 hrs at 40,000 rpm. If salt is added to raise the density to 1.063, and the serum is centrifuged at 30,000 rpm for 12 hrs, all lipoproteins float to the top of the ultracentrifuge tube

with the exception of a high density group, comparable to the α lipoproteins of Cohn and Oncley. The group floating to the top of the ultracentrifuge tube at density 1.063 is designated as the low density group of lipoproteins and includes the β lipoproteins of Cohn and Oncley. All work reported in this paper was limited to studies on the low density group. There are considerable alterations which occur in the high density group in liver damage, however, and these will be referred to in various places below.

The lipoproteins of the low density group are characterized by Svedbergs of flotation, or S_f units. The S_f unit is an arbitrary measurement of the rate of flotation of a particular lipoprotein species. In the low density group, individual lipoprotein species of S_f 2, 4, 6, 8, 10, 13, and 17 have been isolated. In addition, there is a continuous band of lipoproteins above S_f 17 up to S_f 40,000. Above S_f 17, the lipoprotein species are so closely spaced as to be not resolvable in the ultracentrifuge. The higher the S_f of a particular lipopr[illegible] smaller is its hydrated density, the greater is its molecular weight, and the greater is its capacity to scatter light. Lipoproteins of S_f 40,000 correspond to the chylomicrons seen in the microscope.

The constituents of all lipoproteins are cholesterol, phospholipid, neutral fat, and protein. The percentage of each component varies from one lipoprotein species to another and so produces the varying hydrated density responsible for identification in the ultracentrifuge. Lipoproteins of low S_f rate contain more cholesterol, phospholipid, and protein than those of high S_f rate. Each lipoprotein contains a certain percentage of cholesterol in the esterified form (as fractionated by the Schoenheimer-Sperry method), and this percentage decreases with increasing S_f rate. A scheme of lipoprotein composition may be represented as follows:[4]

	S_f 4	S_f 6	S_f 8	S_f 10	S_f 13	S_f 17	S_f 17-40	S_f 40-40,000
Total Cholesterol	30 percent		decreasing					5 percent
Esterified Cholesterol	75 percent		decreasing					0 percent
Phospholipid	25 percent		decreasing					5 percent
Protein	25 percent		decreasing					5 percent
Neutral Fat	absent or very low					increasing		75-85 percent

Children and young adults normally have only lipoproteins of S_f 4 or S_f 6 present in their serum with no other lipoproteins in appreciable concentration. Among older age groups lipoproteins of higher S_f rates may be found. By studying a number of diseased groups of humans, Gofman has drawn up a scale of lipoprotein defects which represent a progressively greater deviation from the normal lipoprotein distribution. The minimal defect observed is the appearance of the S_f 8 lipoprotein. The defect in lipoprotein distribution progresses with the appearance of S_f 10, then S_f 13, and finally a continuous elevation of all lipoproteins up to S_f 40,000. The most extreme defect observed (e.g. xanthoma tuberosum) consists of a reduced S_f 4 or S_f 6 with all other lipoproteins present in large concentration. No lipoprotein of high S_f rate is ever found unless there are some lipoproteins of all the lower S_f rates present. The quantity of the lipoproteins of lower S_f rate may vary, however; the only requirement being that at least some be present.

On eating a meal rich in fat, a transient lipemia is observed even in young adults. This is reflected in a transient rise of lipoproteins whose S_f rate is 60 or more. As this lipemia disappears, there is a stepwise appearance of lower S_f classes down to about S_f 30. No changes in lipoprotein levels below S_f 30 are observed in acute dietary experiments, and lipoproteins below S_f 30 are quite stable in concentration for at least a year. Individuals who maintain large quantities of lipoproteins of the S_f 30-40,000 class in their serum may have a still greater increase in this class after a high fat meal. Normal young adults, however, rapidly clear the dietary lipemia. Measurements of lipoproteins above S_f 30 are influenced by meals and recent dietary events must be taken into account when studying this class.

The normal rabbit is similar to the normal young human in that essentially the only lipoproteins present in the serum are of the S_f 6 or S_f 8 class. No lipoproteins of higher S_f rate are found in any appreciable concentration. Rabbits, however, have a much smaller amount of lipoproteins in their serum as compared to humans, and the normal rabbit shows only a small peak of concentration at S_f 6 or S_f 8.

As the normal rabbit is fed cholesterol, a stepwise series of events occurs which parallels the progressive lipoprotein "defect" outlined above in humans. First the S_f 6 or 8 lipoproteins increase strikingly in quantity, and then lipoproteins of successively higher S_f rate appear. As in humans, no lipoproteins of higher S_f rate appear unless at least some lipoproteins of lower S_f rate are present. Cholesterol feeding in rabbits causes a "spilling over" from the normally occurring S_f 6 or 8 lipoproteins into those of higher S_f rate. With prolonged cholesterol feeding there is eventually a reduction in size of the S_f 6 or 8 lipoproteins and the lipoproteins in greatest concentration may be those of S_f 30 or 40. Feeding Wesson oil with cholesterol accelerates these changes strikingly, although Wesson oil feeding by itself has no effect on lipoprotein levels.[5]

Gofman has shown that lipoproteins of the S_f 12-100 class correlate with atherosclerosis in humans and animals (rabbits, dogs, chicks). Recent work has shown that, in addition, the S_f 0-12 class correlates also with atherosclerosis. In all groups of humans having diseases known to predispose to atherosclerosis (diabetes, hypothyroidism, nephrotic syndrome, hypertension, xanthoma tuberosum), lipoproteins of the S_f 12-100 class are elevated when compared to normals of matched age and sex.[4, 6, 7] Individuals who have had coronary occlusions are significantly segregated from the normal population of corresponding age and sex in that lipoproteins of the S_f 12-100 class are elevated. The lipoprotein segregation is lessened but still evident if the normal and coronary population is matched for the same total serum cholesterol levels. This shows conclusively that the lipoprotein measurements are superior to measurements of cholesterol alone in segregating normal and coronary populations.

There is also a rise in lipoproteins of the S_f 12-100 class with age. This rise is different in the two sexes; males show a sudden striking rise, on the average, between the ages of 25-30. After 30, the quantity of the S_f 12-100 class becomes stabilized at a higher level. In females, there is a slow progressive rise between the ages of 25 to 50 years, and at the end of this time females reach the level attained by males at 30 years. The earlier appearance of elevated levels of

S_f 12-100 lipoproteins in males agrees nicely with the observed higher frequency of atherosclerosis in males in the 30-50 year age group. The sex difference in susceptibility to atherosclerosis is lost beyond 60 years and this also is to be expected from the changes in S_f 12-100 lipoprotein levels with age.

In the alloxan-diabetic cholesterol fed rabbit, Pierce[8] has shown that the serum cholesterol levels actually correlate negatively with atherosclerosis while correlation between atherosclerosis and the S_f 12-40 class is highly positive. The alloxan diabetic rabbit never develops atherosclerosis and the lipoprotein distribution is the same as that of a normal rabbit except for a transient elevation of S_f 5-30 lipoproteins for a few weeks after alloxanization. When the alloxan diabetic rabbit is fed cholesterol, the tendency toward atherosclerosis is strikingly inhibited although serum cholesterol levels may reach enormous concentrations (up to 10,000 mg. percent). Correlation coefficients between atherosclerosis and S_f 12-40 lipoproteins show a Pearson r of 0.8, while the Pearson r between total serum cholesterol and atherosclerosis is -0.3. These rabbits carry almost all of their lipids in lipoprotein classes above S_f 100, and these are not atherogenic. Apparently, in the alloxan-diabetic rabbit, a block occurs at S_f 80-100 with accumulation of lipoproteins above S_f 100, while few lipoproteins below S_f 100 are found in the serum.

The probable role of the liver as an important organ involved in lipid metabolism prompted the study of the effect of impaired liver function on the levels of the lipoproteins in the blood. Carbon tetrachloride (CCl_4) has long been known for its ability to produce a fatty liver and eventual hepatic cirrhosis.[9] This agent has also been shown to be capable of producing an elevation in the total serum cholesterol levels in the rabbit.[10] It was felt, therefore, that the nature of lipid transport (in terms of the lipoproteins involved) in animals treated with carbon tetrachloride might provide information as to certain factors, at least, involved in maintaining blood lipoprotein levels.

Methods

Arbitrarily, the ultracentrifugal diagrams have been analyzed into three broad classes of lipoproteins, the S_f 3-12 class (which include those normally appearing in rabbits), the 12-20 class, and the 20-40 class.

Two general types of experiments were performed. In one group, the effect of carbon tetrachloride injections alone on the serum lipoprotein pattern was determined. In a second group, the combined effect of carbon tetrachloride injection plus cholesterol feeding was studied. Female rabbits, weighing between two and four kg., of the New Zealand white strain were used in all experiments. Seven of 14 animals originally started on carbon tetrachloride injections survived the entire 10 week period of study. Three of these were fed cholesterol throughout the entire period and four were on a normal diet (Albers family style rabbit pellets). The cholesterol food was prepared by dissolving one gm. of cholesterol in 8 cc Wesson oil, which was then thoroughly mixed with 100 gm. rabbit pellets.

After a control blood specimen was drawn, carbon tetrachloride was injected subcutaneously using a dose of one cc per kg. These injections were given twice a week for five and one-half weeks (a total of 11 injections). Blood specimens were obtained at weekly intervals for 10 weeks at which time the experiment was terminated and the animals sacrificed.

Serum was analyzed ultracentrifugally for lipoproteins and by the Schoenheimer-Sperry method for free and total cholesterol.

Results and Discussion

A summary of the results obtained is given in Tables I and II. The data of Table I, which summarize the effect of carbon tetrachloride alone on the serum lipoproteins, shows a consistent trend of events in all four animals. The serum level of the normally occurring S_f 3-12 class of lipoprotein molecules is invariably

Table I

Changes of Cholesterol and Lipoproteins in Rabbits Injected with Carbon Tetrachloride.*

Rabbit	Day from start of Experiment	Lipoproteins-mg %			Cholesterol-mg %		
		3-12	12-20	20-40	Free	Total	F:T
No. 1	0	136	22	2	19	87	0.23
	14	198	59	151	145	350	0.41
	28	253	77	106	-	-	-
	42	242	84	106	64	137	0.47
	56	171	149	50	39	116	0.34
	70	209	24	7	28	116	0.24
No. 2	0	59	4	2	10	62	0.16
	14	198	70	176	139	344	0.40
	28	268	92	139	-	-	-
	42	172	59	26	46	111	0.41
	56	138	85	22	39	111	0.35
	70	128	29	0	19	83	0.23
No. 3	0	51	57	4	14	63	0.23
	14	169	88	132	120	312	0.39
	28	308	62	136	-	-	-
	42	239	103	187	88	194	0.45
	56	77	160	19	27	139	0.20
	70	150	44	22	16	68	0.24
No. 4	0	37	22	0	5	56	0.09
	14	327	92	180	111	281	0.40
	28	272	88	150	127	320	0.40
	42	235	84	31	43	132	0.33
	63	79	9	0	12	59	0.20

* Last injection given on day 39.

Table II

Changes of Cholesterol and Lipoproteins in Rabbits Injected with Carbon Tetrachloride and Fed Cholesterol.*

Rabbit	Day from start of Experiment	Lipoproteins-mg %			Cholesterol-mg %		
		3-12	12-20	20-40	Free	Total	F:T
No. 5	0	24	11	0	7	46	0.16
	14	70	11	66	136	427	0.32
	28	77	264	418	-	-	-
	42	385	154	374	350	660	0.53
	56	396	495	869	444	907	0.49
	70	77	638	1892	478	1700	0.28
No. 6	0	24	26	2	8	51	0.16
	14	297	92	154	181	450	0.40
	28	479	165	270	-	-	-
	42	803	198	193	203	408	0.50
	56	363	220	121	119	297	0.40
	70	396	1353	561	402	1436	0.28
No. 7**	0	55	40	31	9	49	0.18
	14	490	154	160	-	-	-
	28	787	314	303	-	-	-
	42	666	281	281	508	642	0.79
	56	550	1078	704	534	753	0.71
	70	253	>1749	979	680	1971	0.35

* Last injection given on day 39.

** Given no CCl_4 from the second to the third week because of extreme jaundice and cachexia. This was the only animal which became jaundiced.

increased in concentration during the course of carbon tetrachloride injections. Coincident with this increase is the appearance of considerable levels of both the S_f 12-20 and 20-40 classes of molecules. This sequence of changes is the same as that found in a rabbit fed cholesterol. In other words, carbon tetrachloride in rabbits not fed cholesterol is capable of mimicking the lipid and lipoprotein alterations of the serum found when rabbits are fed cholesterol. This represents the synthesis of these "abnormal" giant cholesterol-bearing molecules (of the S_f 12-20 and 20-40 classes) from endogenous sources alone. Parallel with this rise in these three classes of lipoproteins during carbon tetrachloride administration is a concomitant rise in serum cholesterol levels and an increase in the ratio of free to total cholesterol.

When the carbon tetrachloride injections were stopped, an interesting sequence of events occurred during the recovery of the animals from carbon tetrachloride poisoning. As can be seen from Table I, the S_f 20-40 class of molecules returned to relatively low levels within two weeks of the last injection of carbon tetrachloride. At this time the concentration of the S_f 3-12 and 12-20 classes was still elevated, and, in some instances, the S_f 12-20 class showed a moderate increase in concentration. By four weeks after the last injection of carbon tetrachloride, however, the S_f 12-20 class of molecules returned to approximate control levels, although the S_f 3-12 class was still considerably above the original levels before carbon tetrachloride treatment was begun. At the end of the experiment the concentration of the S_f 3-12 class had begun to decrease toward control levels.

During the recovery phase, the ultracentrifugal photographs portray the disappearance of molecules of high S_f value first followed by those of progressively lower S_f value. (See Fig. 1). For example, within the S_f 12-20 class, the species at the higher S_f range decrease before those of the lower S_f range do.

None of these non-cholesterol fed animals showed any atherosclerosis in spite of the transient elevation of the S_f 12-20 and 20-40 classes of molecules during carbon tetrachloride administration. However, the concentration of the S_f 12-40 molecules in rabbits fed cholesterol and developing atherosclerosis is considerably higher than was observed in this experiment. Hence, macroscopic atheroma would not be expected in the carbon tetrachloride injected rabbits in the short experimental period studied.

Table II shows the sequence of events in a carbon tetrachloride injected rabbit being fed cholesterol. The increase in the three classes of molecules analyzed occurs actually more slowly than in a normal rabbit fed cholesterol at the dosage used. However, these animals were rapidly losing weight during the injections and ate very little of the food presented to them. After the last injection of carbon tetrachloride large quantities of the S_f 12-20 and 20-40 classes of lipoproteins developed very rapidly while the S_f 3-12 class remained the same or diminished. The serum cholesterols of this group of animals similarly increased during the carbon tetrachloride injections. After the last injection of carbon tetrachloride the cholesterol levels increased rapidly to very high levels as the animals began to eat more food and consequently increased their intake of cholesterol. All these animals fed cholesterol as well as injected with carbon tetrachloride and which survived the 10 week period showed atherosclerosis.

It should be pointed out that normal untreated rabbits not fed cholesterol consistently show the distribution of lipoproteins as illustrated in "Day 0" (control levels) in the rabbits shown in Table I and II. Repeat samples drawn from a normal rabbit show very little variation from week to week. Consequently the experimental changes reported are in marked contrast to these consistent low levels.

Rabbits injected with alloxan also show a transitory rise of serum cholesterol[11] and of the S_f 3-12, 12-20, and 20-40 and even higher classes of lipoproteins. This substance produces acute liver damage with the development of a fatty liver. In rabbits fed cholesterol fatty livers are also produced. The increase, often to very high levels, in these various classes of lipoproteins which are

present normally in very small quantities may be a reflection of the inability of the damaged liver to handle endogenous or exogenous cholesterol (and other lipids) in the synthesis of the "normal" protein-lipid complexes and in their degradation, by whatever means it occurs. However, the more widespread toxic effects of carbon tetrachloride render it impossible to exclude involvement of other organ systems in the lipoprotein metabolic abnormality.

The changes in free-total cholesterol ratios which occur parallel with the rise and fall in level of the S_f 12-20 and S_f 20-40 classes of lipoproteins provide some information relative to internal structural features of the various lipoproteins. In the animals receiving carbon tetrachloride injections only, the free-total ratio is less than 0.25 at the outset when the lipoproteins present are predominantly in the S_f 3-12 class. As the lipoproteins of the S_f 12-20 and 20-40 classes appear in progressively increasing concentration, the free-total ratio rises significantly. This shows that these classes of lipoproteins differ structurally from the S_f 3-12 class in containing a higher proportion of the cholesterol of the molecule in the nonesterified state as fractionated by the Schoenheimer-Sperry method. These data are in harmony with the data on human lipoproteins which show progressively higher free-total ratios with increasing S_f rate of the lipoprotein species.

In view of these findings in the carbon tetrachloride poisoned rabbit, lipoprotein distribution in humans with cirrhosis of the liver was investigated. Carbon tetrachloride poisoning eventually produces cirrhosis in the experimental animal. The possibility of a similar lipoprotein defect in humans with cirrhosis seemed likely, although the etiology of the human disease is by no means as clear cut as in the experimental animal.

In addition, a clinical impression has long been in existence indicating a lesser degree of atherosclerosis in persons with cirrhosis. From an analysis of an extensive autopsy experience, Wilens[12] has presented substantial evidence that there is no significant difference in the extent of atherosclerosis in cirrhotics as compared with other individuals of comparable status and of

equivalent age and sex distribution. In view of this controversy, any evidence as to change in the levels of the S_f 10-20 class of lipoproteins in cirrhotics would shed light on the status of atherosclerosis in these patients, since the S_f 10-20 class of lipoproteins correlates well with atherosclerosis.

Methods and Results

Thirty-four patients were studied, ranging in age from 17 to 72 years including both males and females. The blood serum was analyzed for lipoproteins of the S_f 10-20 class ultracentrifugally. Table III summarizes the pertinent clinical and laboratory data and the S_f 10-20 lipoprotein levels. Figure 2 is a plot comparing the distribution of levels in the cirrhotic with the levels in clinically normal individuals of comparable age and sex distribution. It is evident that cirrhotics have at least as high, and possibly even higher, levels of the S_f 10-20 class of molecules as do clinically normal individuals of corresponding age and sex. However, the difference between these two groups is not significant so no further refinement can be obtained from the data of Fig. 2.

No correlation between the levels of the S_f 10-20 class of molecules and any of the clinical or laboratory findings recorded in Table III could be established.

Since the level of the S_f 10-20 class of lipoproteins has been correlated with atherosclerosis and since the level of such molecules is not significantly different in patients with chronic hepatitis as compared with "normals", one would anticipate that such patients might show the same degree of atherosclerosis as found in the general population. These findings would support Wilen's observations indicating that patients with chronic hepatitis are not protected against atherosclerosis.

From this study, it is obvious that the cirrhotic human does not behave like the carbon tetrachloride poisoned rabbit insofar as lipoprotein distribution is concerned. Were the human cirrhotic to behave like the CCl_4 poisoned rabbit, one would expect striking elevation of the S_f 10-20 class. This disparity between humans and rabbits

Table III

—*Clinical Data on the Cirrhotic Patients Used in This Study*[a]

Case No.	Sex	Age	Ascites	Liver	Spleen	Icteric Index	Cephalin Flocculation	Albumin-Globulin Ratio	Thymol Turbidity	RBC Millions	Hemoglobin	Diagnosis	Sr10-20	Remarks
				cm.	*cm.*		*48 hours*	*Gm. per cent*			*Gm. per cent*		*mg. per cent*	
1	M	40	yes			2.1	++++	2.5/3.3		2.5	8.0	Cirrhosis with hepatoma (biopsy)	11	Expired 2 days after test
2	M	48	yes			8.6	++++	2.9/3.2	2.1	3.6	10.0	Cirrhosis	37	Expired 12 days after test
3	M	27	no	3		12.0	+	2.6/3.3	3.8	3.3	10.0	Cirrhosis	81	Expired 6 days after test
4	M	57	no	3		47.0	+	2.8/2.9	2.3	3.1	7.0	Cirrhosis	95	
5	F	49	yes	5		21.0	++++	2.6/2.8	8.3	2.7	7.8	Cirrhosis	68	
6	F	42	no			44.0	++++	2.0/2.9	3.3	3.7	12.3	Severe fatty infiltration of liver (autopsy)	110	Expired 5 days after test
7	F	43	no	5	4	25.0	++++	3.0/3.5			10.0	Cirrhosis and psoriasis	73	
8	F	49	yes	4		55.0	+++	3.0/3.0	2.5	3.0	7.0	Cirrhosis	31	
9	M	43	yes			9.0	+++	2.6/2.9			9.5	Cirrhosis	42	
10	M	65	no	4		5.0	+	2.3/3.7		2.7	9.0	Cirrhosis (biopsy)	53	Expired 10 days after test
11	M	55	no	3		82.0	++++	2.5/4.7	8.7	3.9	14.0	Cirrhosis	50	Expired 13 days after test
12	M	48	no	3		80.0	++++	3.1/5.0	8.0	4.7	12.0	Cirrhosis	26	
13	F	35	no	12	5	56.0	+++	2.3/3.4	4.4		9.0	Cirrhosis	132	
14	F	72	yes	3		15.2	±	2.8/1.9		4.6	15.0	Cirrhosis (biopsy)	7	Expired 18 days after test
15	F	54	yes	5		6.1	++++	2.8/3.0		4.1	12.5	Cirrhosis	35	Expired 12 days after test
16	M	47	yes	2		32.0	++	3.2/4.1		2.9	11.0	Cirrhosis (biopsy)	64	Expired 32 days after test
17	M	55	yes	4		12.3	+	2.9/2.8	10.5		8.4	Cirrhosis	48	
18	M	60	no	4		13.2	++++	2.8/3.3	3.1	3.7	11.0	Cirrhosis	53	Expired 5 days after test
19	M	52	no	3			0		1.0	3.3	9.8	Cirrhosis (biopsy)	64	
20	M	47	no			60.0	+++	2.7/4.0	12.0		11.0	Cirrhosis (biopsy)	26	
21	F	69	no	2							12.3	Cirrhosis (biopsy)	33	
22	M	55	yes	3		4.0	+++	2.2/3.3	8.0		10.5	Cirrhosis	73	
23	M	67	no		1		++				12.6	Cirrhosis (biopsy)	84	
24	F	70	yes			39.0		2.9/3.6			11.0	Cirrhosis	29	Expired 120 days after test

MU-5826

Table III
(Continued)

—*Concluded*

Case No.	Sex	Age	Ascites	Liver	Spleen	Icteric Index	Cephalin Flocculation	Albumin-Globulin Ratio	Thymol Turbidity	RBC Millions	Hemoglobin	Diagnosis	Sr 10-20	Remarks
				cm.	*cm.*		*48 hours*	*Gm. per cent*			*Gm. per cent*		*mg. per cent*	
25	M	50	yes	2								Cirrhosis	53	
26	M	46	yes	2		8.0	++++	2.4/3.4	10.0		13.0	Cirrhosis	66	Expired 4 days after test
27	M	54	yes	3		42.0	++	2.6/1.8		4.5	11.5	Cirrhosis (biopsy)	48	
28	F	53	yes			21.0	+++	2.5/3.7			13.0	Cirrhosis	70	
29	M	65	yes	6		14.0	++++	2.8/2.7		2.3	8.9	Cirrhosis	18	
30	M	72	yes			41.0	++++	2.1/3.9		4.1	14.5	Cirrhosis	51	
31	M	55	no	4		8.0	++	3.4/2.6			13.0	Cirrhosis	33	
32	M	30	yes	3		21.0	++++	2.0/4.5		3.1	14.0	Cirrhosis	26	Expired 41 days after test
33	M	21	no			47.5	0	4.0/3.5	5.7	5.6	15.0	Infectious hepatitis	150	
34	M	17	no			15.2	++++	3.7/2.7		5.3	13.0	Infectious hepatitis	68	

* Two patients with infectious hepatitis are included.

MU-5827

is not surprising since the human disease is a chronic process lasting many years and related to faulty nutrition. The CCl_4 poisoned rabbit suffers from acute liver damage, whose relationship to the human disease is doubtful.

It is of great interest that two male patients, 17 and 21 years of age, in the active phase of infectious hepatitis showed very high levels of the S_f 10-20 class of lipoproteins. This is in sharp contrast to the rarity of such high levels in normals of this age group. Accordingly, lipoprotein distribution was investigated in a group of patients with infectious and serum hepatitis.

It is well established that alterations in the serum lipids occur in viral hepatitis.[13,14,15] There is a tendency for the neutral fats, cholesterol, and phospholipids to rise and the appearance of this hyperlipemia has been associated with the onset of jaundice. The increase in phospholipids has been attributed to a rise in phospholipid lecithin, with sphingomyelin and cephalin remaining unchanged.[16]

The most striking lipid alteration, however, is in the change of the ratio of free to esterified cholesterol during the acute phase of viral hepatitis.[17,18,19] This change may or may not be associated with a change in total cholesterol but an absolute fall in cholesterol esters with an absolute rise in free cholesterol occurs. The minimum cholesterol ester concentration occurs usually in the 2nd to 3rd week of the disease and returns to normal values with recovery. In patients who go on to develop chronic liver disease as a result of viral hepatitis, the free to ester cholesterol ratio returns to normal after the initial changes in the acute phase.[20]

Since over 95 percent of the lipids in serum can be accounted for by the amounts of lipoproteins present, any change in lipid content of serum must be reflected in a change of one or more groups of lipoproteins. In view of the change of the free to ester cholesterol ratio in serum in viral hepatitis an appropriate change in lipoprotein distribution would be expected to account for the predominance of cholesterol in the free form in this disease.

Methods

Thirty-three patients with infectious hepatitis and fifteen patients with serum hepatitis were studied. Blood samples were drawn at the Oakland Naval Hospital and sent to Donner Laboratory for ultracentrifugal analysis. On many of these patients repeat blood samples were obtained during the course of their disease, and a total of ninety-seven ultracentrifugal analyses were done. The following liver function tests were performed on the same day the blood for lipoprotein studies was drawn: Icterus index, cephalin-cholesterol flocculation, colloidal red, thymol turbidity (pH 7.55), albumin-globulin ratio, gamma globulin, zinc turbidity, total lipids, and serum bilirubin (both direct and total).

Two of these tests are stated to be a measure, at least in part, of lipid or lipoprotein derangement. The thymol turbidity test has been shown by MacLagen to depend on precipitation of material containing lipids and protein.[21] Furthermore, sera which give a positive reaction to this test become negative when the lipids are first extracted.[22] The turbidity of the thymol reagent is stated to depend on serum lipids and abnormal lipid-protein complexes migrating with the beta globulin fraction of serum. The gamma globulin fraction is also important for the reaction, and in viral hepatitis, the thymol turbidity is reported to parallel alterations in serum lipids initially and gamma globulin alterations during late convalescence.[23] The total lipid method used was that of Kunkel, Ahrens, and Eisenmenger and depends on turbidity in serum produced by the addition of phenol.[24] The authors state this test has a coefficient of correlation of 0.6 with the lipid carbon method of Van Slyke and co-workers.

The differential diagnosis between serum or infectious hepatitis was made on the basis of transfusions of whole blood or plasma within recent months and with an appropriate incubation time between the transfusion and the appearance of jaundice.

The blood specimens were analyzed for lipoproteins of the S_f 0-12, 12-20, 20-100, and 100-400 classes using corrections in area measurements recently described by Gofman[25] et al. for self-slowing effects (standard S_f rates). In addition, the corrected flotation rate of the major peak in the S_f 0-12 class was measured and is included in the tabulation of data.

Results

Table IV is a summary of correlation coefficients (Pearson r) between each lipoprotein class and each of the liver function tests. Serial samples on the same patient are included in this analysis. Also reported in this table is the correlation coefficient between the flotation rate of the major peak in the S_f 0-12 class and the icterus index. The number of cases involved and the significance of the various correlation coefficients is tabulated.

It is apparent from Table IV that the most striking correlations exist between the various lipoprotein classes and the icterus index. The icterus index correlates with a high degree of significance with the S_f 12-20 class and to a lesser, but still significant, degree with the S_f 0-12 and S_f 20-100 classes. There is a negative correlation between the S_f 100-400 class and icterus index significant to the five percent level. There is also a very significant correlation between the flotation rate of the major peak and the icterus index. The cases studied were separated into two groups: those with a normal icterus index (of 10.4 or less) and those with an elevated icterus index (of 10.5 or more). Correlation coefficients were again calculated for lipoproteins and icterus index in each of these two groups. Table IV shows the result, and it can be seen that no significant correlation between lipoproteins and icterus index occurs in those patients with an icterus index of 10.4 or less. The correlation coefficients, moreover, have become negative with the S_f 0-12 and 20-100 classes of lipoproteins in contrast to the situation where all cases are included, further demonstrating the lack of relationship between lipoproteins and icterus index in this group. In those patients whose icterus index is elevated (i.e., 10.5 or more), a highly significant positive correlation with the S_f 12-20 class and a highly significant negative correlation with the S_f 100-400 class exists. This information is taken to indicate that jaundice is either associated with or directly responsible for the lipoprotein changes in infectious and serum hepatitis.

Table IV

Correlation coefficients (Pearson r) between various lipoprotein classes and liver function tests. N is the number of cases studied, and P is the probability of significance at the 1 percent or 5 percent level. In addition, the correlation coefficient between the flotation rate (S_f) of the major peak in the S_f 0-12 class and the icterus index is included.

Liver Function Test	S_f 0-12			S_f 12-20			S_f 20-100			S_f 100-400		
	r	N	P	r	N	P	r	N	P	r	N	P
Icterus Index	0.241	87	5%	0.475	87	1%	0.220	87	5%	-0.232	87	5%
Thymol Turbidity	0.151	90	--	0.203	90	--	0.184	90	--	0.019	90	--
Albumin / Globulin ratio	-0.034	80	--	-0.196	80	--	-0.139	80	--	0.208	80	--
Total Lipids	0.196	58	--	0.219	58	--	0.331	58	1%	0.141	58	--
Gamma Globulin	0.108	56	--	0.131	56	--	0.215	56	--	-0.106	56	--
Zinc Turbidity	-0.084	58	--	-0.051	58	--	0.060	58	--	-0.116	58	--
Cephalin Cholesterol Flocculation	0.041	90	--	0.109	90	--	-0.069	90	--	-0.174	90	--
Colloidal Red	0.160	38	--	0.325	38	5%	0.106	38	--	-0.165	38	--
Bilirubin Direct	0.115	87	--	0.378	87	1%	0.212	87	5%	-0.243	87	5%
Bilirubin Total	0.171	87	--	0.379	87	1%	0.214	87	5%	-0.237	87	5%
Icterus (cases selected for 10.4 or less) Index	-0.080	15	--	0.191	15	--	-0.276	15	--	-0.173	15	--
Icterus (cases selected for 10.5 or more) Index	0.156	72	--	0.422	72	1%	0.133	72	--	-0.338	72	1%

Variables	r	N	P
S_f 0-400 vs. thymol turbidity	0.210	90	5%
S_f 0-400 vs. total lipids	0.302	58	5%
S_f major peak vs. icterus index	0.465	87	1%

The correlation coefficients between the serum bilirubin levels (both direct and total) are essentially the same as for the icterus index. This is not surprising in view of the similarity of these measurements which are all directed toward quantifying the degree of jaundice.

No individual class of lipoproteins correlates significantly with the thymol turbidity test. However, there is a correlation, significant at the five percent level, between the entire S_f 0-400 class of lipoproteins and thymol turbidity readings. If the thymol turbidity test is associated with serum lipids only during the acute phase of the disease, and dependent on gamma globulin levels later in the disease, this association may have been diluted by using the entire group of patients many of whom were in the later convalescent stages of hepatitis.

The total lipid determination of Kunkel, Ahrens, and Eisenmenger goes not correlate significantly with any lipoprotein class with the exception of the S_f 20-100 class, which correlates significantly at the one percent level. There is also a correlation between the entire S_f 0-400 class and total lipids significant to the five percent level. This suggests that, of these lipoprotein classes, the S_f 20-100 class is the one most associated with the lipids precipitated by phenol in acute hepatitis, but no further evidence for this is available at this time.

Correlation coefficients with the gamma globulin level, zinc turbidity, and cephalin cholesterol flocculation test were uniformly without significance. Correlation coefficients between lipoproteins and albumin-globulin ratios were all without significance, but it is interesting that the sign of the Pearson r was the reverse of that found with icterus index in all four classes of lipoproteins. This is to be expected since this is the only liver function test whose numerical value increases with recovery. The colloidal red test correlates at the five percent level of significance with the S_f 12-20 class of lipoproteins, but no significant correlation is found for any of the other lipoprotein classes.

Table V presents the means and standard deviations of the four classes of lipoproteins in all patients with hepatitis. Repeat samples on the same patient were excluded from this calculation, and in cases of serial samples, the first one obtained was used.

Table V

Means and standard deviations of various classes of lipoproteins in patients with infectious and serum hepatitis. Lipoproteins expressed in mg%. The mean and standard deviation of the flotation rate (S_f) of the major peak in the S_f 0-12 class is also presented, and is expressed in Svedbergs of flotation. For comparison with normal values obtained from healthy persons of matched age and sex, the "students" t ratio is listed. P is the probability of significant difference at the 1% or 5% level. N is the number of cases studied, and σ is the standard deviation.

	S_f 0-12			S_f 12-20			S_f 20-100			S_f 100-400		
	Mean	σ	N	Mean	σ	N	Mean	σ	N	Mean	σ	N
Normals	306.9	62.0	48	47.7	17.2	48	88.0	38.3	48	50.6	31.1	48
Hepatitis all cases	374.5	98.3	48	98.6	60.3	48	114.2	55.3	48	32.5	26.7	48
Infectious Hepatitis	375.6	97.4	33	84.9	44.8	33	105.7	46.6	33	32.7	27.3	33
Serum Hepatitis	371.8	100.6	15	128.8	76.6	15	133.3	66.8	15	32.3	25.3	15
Hepatitis all cases	(Icterus index 10.4 or less)* 286.3	86.9	15	49.5	29.6	15	73.2	28.4	15	22.8	15.0	15
Hepatitis all cases	(Icterus index 10.5 or more)* 365.8	95.2	72	91.6	55.8	72	114.0	56.4	72	40.0	30.5	72

	Mean	σ	N
S_f major peak Normals	7.39	0.95	47
S_f " " Hepatitis all cases	8.41	1.20	48

* Repeat studies on same patient included.

Variables			t	P	
Normal	S_f 0-12 x	Hepatitis all cases S_f 0-12	3.974	1%	
"	S_f 12-20 x	" " " S_f 12-20	5.564	1%	
"	S_f 20-100 x	" " " S_f 20-100	2.659	1%	
"	S_f 100-400 x	" " " S_f 100-400	3.00	1%	
"	S_f 0-12 x	" " " S_f 0-12	0.829	--	Icterus index 10.4 or less
"	S_f 12-20 x	" " " S_f 12-20	0.216	--	
"	S_f 20-100 x	" " " S_f 20-100	1.568	--	
"	S_f 100-400 x	" " " S_f 100-400	4.593	1%	
"	S_f 0-12 x	" " " S_f 0-12	4.046	1%	Icterus index 10.5 or more
"	S_f 12-20 x	" " " S_f 12-20	6.203	1%	
"	S_f 20-100 x	" " " S_f 20-100	2.974	1%	
"	S_f 100-400 x	" " " S_f 100-400	3.765	1%	
Infectious Hepatitis					
"	S_f 0-12 x	Serum Hepatitis S_f 0-12	0.078	--	
"	S_f 12-20 x	" " S_f 12-20	2.00	--	
"	S_f 20-100 x	" " S_f 20-100	1.40	--	
"	S_f 100-400 x	" " S_f 100-400	0.0541	--	
Normal	S_f major peak x	Hepatitis all cases S_f major peak	4.636	1%	

Means and standard deviations of normals are also presented in Table V. The normals were matched with the hepatitis cases for age and sex; the rather low mean values for lipoproteins in the normal individuals is explained since most cases were in the 18-25 year group. The mean and standard deviation of the flotation rate of the major peak (in the S_f 0-12 class) is presented for both the normal and the hepatitis group. The "t" values and the probabilities of significant difference were calculated for each class of lipoproteins and for the major peak S_f rate in the normal group as compared with the hepatitis group. It can be seen from Table V that there is a highly significant difference for each lipoprotein class and for the major peak S_f rate between values found in hepatitis cases as compared with matched normals. The S_f 0-12, 12-20, 20-100 classes of lipoproteins and the S_f rate of the major peak are all significantly higher; the S_f 100-400 class of lipoproteins is significantly lower.

All hepatitis cases were split into two groups: serum hepatitis and infectious hepatitis. The mean and standard deviation of each class of lipoproteins was again calculated and the levels in each form of hepatitis compared. The "t" values and the probabilities of significant difference are presented in Table V, and it can be seen that no significant difference in any class of lipoproteins was found. In view of the similarity of these two diseases, it is not surprising that lipoprotein changes should be so similar in each.

All hepatitis cases were split in a second manner into two groups: those having an icterus index of 10.4 or less and those having an icterus index of 10.5 or more. In this study serial samples were included as well. The means and standard deviations of the four lipoprotein classes were calculated in each group and compared with the normal group. It can be seen from Table V that those cases with a normal icterus index are not significantly different from the normal population with the exception of the S_f 100-400 class, which is significantly lower. However, those cases with an elevated icterus index are significantly different in all classes of lipoproteins (higher in S_f 0-12, 12-20, and 20-100; lower in S_f 100-400) when compared with normals. This is taken to suggest that the lipoprotein levels return

to normal values as the icterus returns to normal and corroborates the significant correlations between the icterus index and each lipoprotein class reported above.

Figure 3 is a composite graph showing the change of the four classes of lipoproteins, the S_f rate of the major peak, the icterus index, thymol turbidity test, and cephalin-cholesterol flocculation during the course of the disease. Each point in this graph is the mean of all the measurements of any given test performed in each week of the disease. The onset of jaundice is taken as "Day 1"; no patients were studied before this event took place. The graph is only extended to 9 weeks; several patients were studied later in the course of the disease, but they were necessarily the more chronically ill and remained in the hospital for a longer lime. In order to minimize the effect of the chronically ill patients becoming relatively predominant in the later phase of the disease, an arbitrary time limit of the course of the disease was set at the ninth week. Most individuals were well over their disease at this time. It can be seen from Fig. 3 that the lipoproteins of the S_f 0-12, 12-20, and 20-100 classes decrease progressively as the weeks of illness pass, as does the S_f rate of the major peak. The S_f 100-400 does not show this trend and, if anything, tends to rise ruring the course of the disease. The icterus index and thymol turbidity show a progressive diminution during the course of the disease, with the icterus index falling more rapidly.

Figure 4 is a graph of the changes occurring in four individual patients followed serially through at least part of their illness. In this figure there are shown the lipoprotein levels, the S_f rates of the major peak, and the icterus index, thymol turbidity, and cephalin-cholesterol flocculation. One patient suffered a relapse after leaving the hospital; the other three are well to date. The changes shown in Fig. 4 illustrate individually the course of events summarized in Fig. 3.

Discussion

In infectious and serum hepatitis, there is a very significant increase in lipoproteins of the S_f 0-12, 12-20, and 20-100 classes and a very significant reduction in the S_f 100-400 class when compared with normal individuals of matched age and sex. These changes in lipoprotein levels are strongly associated with changes in the icterus index. When hepatitis patients with normal icterus are compared with a normal healthy group of individuals the significant difference disappears except for the S_f 100-400 class of lipoproteins. In patients with elevated icterus, highly significant differences in all classes of lipoproteins remain. Correlation coefficients between icterus index and lipoproteins are significant for each class of lipoproteins studied. In individuals with normal icterus, this correlation disappears (except for the S_f 100-400 class). In individuals with elevated icterus, there is a very significant positive correlation between icterus index and the S_f 12-20 class and a very significant negative correlation between the S_f 100-400 class and the icterus index. Serum bilirubin levels (both direct and total) show the same correlation with lipoproteins as does the degree of icterus. No clear explanation for this derangement in lipoproteins can be made at this time. It is possible that bilirubin or some other substance exerts a direct effect on lipoproteins in the serum, but it is equally possible that the lipoprotein changes reflect the underlying hepatic disturbance. Since the lipoprotein levels return toward normal in a pattern paralleling the return of icterus toward normal, nearly simultaneous hepatic disturbance seems probable.

The change in the proportion of free to esterified cholesterol in acute hepatitis with the esterified cholesterol dropping to very low values deserves comment in relation to the changes observed in lipoprotein levels. This decrease is an absolute one, with the total cholesterol remaining unchanged or showing a tendency to increase. In addition to the lipoproteins reported in this study, the high density lipoproteins must be considered which are markedly altered in acute hepatitis.[26] The high density group accounts for 25-50 percent of the serum cholesterol and of this cholesterol, approximately 80 percent is in the esterified form. This group is greatly diminished or

completely suppressed in the acute phase of hepatitis, and consequently a large contribution of esterified cholesterol disappears from the lipoprotein scene. Lipoproteins of the S_f 0-12 class contain appreciably more cholesterol in the free form as compared to the high density group, and the percent of free cholesterol per molecule increases with increasing S_f rate. From the reduction of the high density group of lipoproteins (80 percent of whose cholesterol is esterified) together with an increase in the S_f 0-12, 12-20, and 20-100 classes one should expect an absolute reduction in esterified cholesterol in this disease. However, a reduction in esterified cholesterol below 50 percent is difficult to visualize. The S_f 100 lipoprotein molecule contains its cholesterol approximately 50 percent in the esterified form. Lipoproteins greater than S_f 100 are reduced in this disease, and therefore their contribution must be neglected even though they possess most of their cholesterol in the free form.

Unfortunately, no cholesterol measurements are available on the data reported here. Without individual measurements of both cholesterol and lipoproteins no certain conclusions may be reached. However, the only explanation of a level of esterified cholesterol which is below 50 percent and a lipoprotein distribution as reported above is to assume a change in composition of the individual lipoproteins in this disease as compared with normals. This change may be a complete re-shuffling of the lipoprotein molecule with all components altered, or it may be the same basic structure with the addition of cholesterol in the free form, and other components remaining the same.

The change in the flotation rate of the major peak is of importance in this consideration. The flotation rate is significantly high in the acute phase of hepatitis, correlates well with the icterus index, and returns to normal paralleling the return to normal of the icterus index during convalescence. It is conceivable that, in some fashion, free cholesterol becomes associated with the lipoproteins constituting the major peak which increases their buoyancy and their S_f rate.

Further experiments are in progress to investigate these possibilities, viz., chemical analysis of isolated lipoproteins from patients with acute hepatitis and in vitro studies of the effect of serum from jaundiced individuals on lipoproteins.

In connection with the changes observed in viral hepatitis, the observations of Gofman on lipoprotein changes in biliary cirrhosis may be mentioned.[27] In this disease, as in viral hepatitis, there is a striking elevation of free serum cholesterol with a reduction in cholesterol esters. The lipoprotein distribution in biliary cirrhosis is characterized by the appearance of enormous quantities of the S_f 0-12 and 12-20 classes in the serum, and the essentially complete absence of lipoproteins in the S_f 100-400 class. These changes are more marked in biliary cirrhosis than viral hepatitis, but the same type of change is found in both. The similarity of lipoprotein change in these two forms of liver disease suggests that there is a single type of lipid metabolic defect common to both and that this defect is even more apparent in biliary cirrhosis than viral hepatitis.

Other changes in serum lipids are consistent with the lipoprotein alternations in this disease. The tendency toward elevated total cholesterol, phospholipid, and neutral fat values in the serum is consistent with the increase in lipoproteins of the S_f 0-100 class. Since the high density group of lipoproteins is reduced in this disease, it is unnecessary to expect an invariable increase in serum lipids. The total values of cholesterol, phospholipid, and neutral fat in the serum must reflect a balance in these two different lipoprotein changes.

The changes in lipoprotein levels do not segregate infectious hepatitis from serum hepatitis, and the lipoprotein changes in these two forms of viral hepatitis are identical.

No individual class of lipoproteins correlates significantly with the thymol turbidity test; the entire S_f 0-400 class does correlate with a coefficient significant at the five percent level. The thymol turbidity test, if it is to correlate with any lipoprotein group, must correlate with the lipoproteins studied here. No date is available as yet on the relation of this measurement to high density lipoproteins or to lipoproteins above S_f 400. The latter are generally present in

very trivial concentration and, if present in any quantity, would render the serum very milky and turbid. This phenomenon was never observed in the patients studied. The high density class of lipoproteins, being diminished or absent in acute hepatitis, seems unlikely to contribute to this measurement. It seems probable then that the S_f 0-400 represents the lipoprotein group responsible for the thymol turbidity measurement, but that no class within this group is of special importance.

It is conceivable that, if one selected cases with high thymol trubidity readings, one might find a significant correlation with one of the individual classes of lipoproteins. Similarly, a selection of patients in the early weeks of the disease might afford better correlation in view of the observation that the thymol turbidity measurement is associated with serum lipids in the acute phase of the disease, and with the gamma globulin level during convalescence.

Only the S_f 20-100 class correlates significantly (at the one percent level) with the total lipid determination described by Kunkel, Ahrens, and Eisenmenger. This test involves the precipitation of lipids by phenol in a hypertonic salt solution. This relationship suggests that the S_f 20-100 class of lipoproteins is the principal contributor to lipids precipitated by phenol. The S_f 0-400 class also correlates with the total lipid determination but with a coefficient significant only at the five percent level. The greater significance of the correlation between S_f 20-100 and total lipids may be interpreted to mean that the correlation of the S_f 0-400 is accounted for by its S_f 20-100 constituent. For reasons mentioned above, neither the high density group nor lipoproteins above S_f 400 would be expected to contribute to the total lipid measurement in acute hepatitis.

The albumin-globulin ratio shows a small negative correlation with the S_f 0-12, 12-20, 20-100 classes of lipoproteins, and a positive correlation with the S_f 100-400 class. While none of these correlations is significant to the five percent level, the sign of each correlation was the reverse of that found with the icterus index. Since this liver function test increases with recovery, the reversal of the sign of the correlation coefficient is to be expected.

There is no significant correlation between any class of lipoproteins and the gamma globulin determination, zinc turbidity, or cephalin-cholesterol flocculation. The S_f 12-20 class of lipoproteins correlates significantly with the colloidal red determination, although none of the other lipoprotein classes does so. No obvious explanation for this relationship can be made.

There is some similarity between humans with acute hepatitis or biliary cirrhosis and the CCl_4 poisoned rabbits with respect to the lipoprotein changes found. In all these situations, the S_f 0-40 class of lipoproteins is markedly elevated. The normal rabbit has essentially no lipoproteins above S_f 100 and any reduction of this class by CCl_4 could not be observed. The high density group of lipoproteins appears to be in very small concentration in the normal rabbit although the status of this group of lipoproteins has not been intensively investigated in this animal. At least in the lipoproteins measured above, there is a similar direction of change in lipoprotein distribution under these three conditions.

Patients with cirrhosis do not appear to have any striking elevation of the S_f 10-20 lipoproteins and so do not follow the lipoprotein pattern of acute liver damage. Several of the cirrhotic patients studied were in terminal condition, and it has been reported that all serum lipids fall strikingly with liver failure. Possibly patients in liver failure, with reduced serum lipids, diluted any elevation of lipoproteins which might be found if only the cirrhotics who were less ill were studied. However, the absolute increase in free serum cholesterol found in acute hepatitis is found to a much less extent in cirrhotics. Similarly, the tendency toward hyperlipemia in acute hepatitis is not reported in cirrhosis. It is of interest that the free serum cholesterol which rises in acute hepatitis returns to normal values after the acute episode is passed. If the patient goes on to develop chronic liver disease after viral hepatitis, the free cholesterol does not remain elevated but returns to normal levels and remains normal. It would appear, then, that chronic liver disease does not produce the lipoprotein disturbance found in acute hepatitis at least in the low density group. There is evidence that the high density

group is reduced in both cirrhosis and acute hepatitis, and this may represent a different facet of lipid disturbance common to both types of liver disease.

The similarity of biliary cirrhosis to acute, viral hepatitis is noteworthy. The "obstructive" element is common to both types of liver disease, and liver function tests elevated by bile duct obstruction are elevated in these forms of liver disease. The lipoprotein distribution in patients with pure bile duct obstruction (stone, pancreatic cancer) has not been investigated, but might clarify any relation between obstruction and elevation of the low density lipoproteins of the S_f 0-40 class. It is well known that there are large amounts of free cholesterol in the bile. Obstruction of bile flow might force this cholesterol into the blood stream, as is the case with bile pigments, but this relation is only empirical at the present.

In addition to the types of liver damage reported above, alloxan poisoning and cholesterol feeding produce the same type of changes in lipoprotein distribution with some modifications. Alloxan poisoning produces changes exactly comparable to CCl_4. Lipoproteins appear and regress with recovery in the same fashion. Cholesterol feeding, as described earlier, also produces this same type of change, and with cessation of cholesterol feeding, recovery occurs as with CCl_4. Cholesterol feeding, however, will go on to produce much greater quantities of lipoproteins than CCl_4 poisoning. Also cholesterol feeding produces lipoproteins above S_f 100 eventually, and these lipoproteins are never found after CCl_4 injection in the rabbit. A fatty liver is found in CCl_4 and alloxan poisoning, and also after cholesterol feeding. Perhaps this is the common denominator for this type of lipoprotein defect found in these three experimental conditions in the rabbit. Fatty livers are not reported in humans with acute hepatitis, however; hence the analogy can not be extended too promiscuously.

The alloxan diabetic rabbit fed cholesterol and the rabbit injected with cortisone show totally different types of lipoprotein defects.[28] The cholesterol fed alloxanized rabbit (not to be confused with acute alloxan poisoning) exhibits a block at approximately the S_f 80-100 class. Lipoproteins above S_f 100 accumulate in tremendous quantities, while those below remain at assentially normal levels.

As mentioned above, this fact may be used to explain the resistance to atherosclerosis in these animals. The cortisone injected rabbit develops a block at the S_f 40 species. With continuing injections of cortisone, huge quantities of lipoproteins appear above S_f 40, while lipoproteins below S_f 40 are reduced to essentially nothing.

Lipoproteins may be strikingly increased by radiation,[29] excess hemorrhage, diarrhea, or pneumonia in the rabbit. The type of change found in these conditions has not been satisfactorily investigated to describe it accurately. Radiation may produce a cortisone-like rise in lipoproteins of high S_f rate, or a CCl_4-like rise in lipoproteins of low S_f rate.

One common factor to all types of experiments designed to elevate rabbit lipoproteins is the pattern of recovery once the causative agent is removed. Recovery from CCl_4 poisoning, alloxan poisoning, cortisone administration, radiation and cholesterol feeding follows a definite course. Lipoproteins of highest S_f rate disappear first, intermediate classes disappear next, and the normally occurring S_f 6-8 returns to normal levels last. In consequence of this observation, an experiment was designed to critically test the pathway of normal lipoprotein metabolism by injecting isolated lipoproteins of various classes into normal rabbits. The individual classes of lipoproteins were isolated in large quantity from the serum of cholesterol fed rabbits, and in this way the fate of isolated classes of lipoproteins could be determined by serial studies on the recipient rabbit's serum.

Methods

Five classes of lipoproteins were isolated: S_f 5-15, 15-20, 20-100, 100-400, and 400 +.

The S_f 5-15, 15-20, and 20-100 classes were obtained from the sera of normal rabbits fed a cholesterol containing diet for two months. This diet was prepared by dissolving one pound of cholesterol in 1500 cc Wesson oil with gentle heating. The Wesson oil-cholesterol mixture was added to 100 pounds rabbit pellets (Albers family style) and thoroughly mixed. The rabbits were fed this diet and water ad lib. At the end of

two months on this diet, the rabbits were exsanguinated by jugular puncture and the serum was obtained. Nine cc of serum were placed in each of 20 tubes in the Spinco Model L preparative ultracentrifuge. Centrifugation was performed at 30,000 rpm for 24 hrs. At the end of this time, lipoproteins whose S_f rate was 20 or greater had floated to the top cc of the centrifuge tube forming a densely turbid layer. Since lipoproteins whose S_f rate is greater than 100 were in relatively low concentration, the lipoproteins from the top cc were designated as the S_f 20-100 class. The serum proteins and high density lipoproteins had sedimented to the bottom 3 cc of the tube, and on top of these was a 1 cc yellowish opalescent layer containing the S_f 5-15 class of lipoproteins. The S_f 15-20 class of lipoproteins does not migrate appreciably in serum whose density is unaltered, and these lipoproteins were found in a relatively clear area averaging 3.5 cc beneath the top cc and above the layer containing the S_f 5-15 class. Each of these three classes of lipoproteins was removed from the centrifuge tubes by pipetting and carefully separated from each other. The serum proteins and high density lipoproteins at the bottom of the centrifuge tube were discarded. The lipoproteins of the S_f 20-100 class and of the S_f 5-15 class were nine times as concentrated in the isolated fractions as compared to their concentration in the original cholesterol fed rabbit serum. In order to obtain a satisfactory loading of lipoproteins in the blood of a normal rabbit, 10 cc of concentrated lipoproteins of each class were injected. 4 or 5 cholesterol fed rabbits had to be exsanguinated to obtain 180 cc serum, from which 20 cc of the S_f 20-100 class and 20 cc of the S_f 5-15 class were isolated. This quantity of lipoproteins was sufficient for the injection intravenously into two rabbits of each class of lipoproteins. The S_f 15-20 class of lipoproteins was not concentrated by centrifugation, and approximately 70 cc of this class of lipoproteins was obtained from 180 cc serum. 35 cc of the S_f 15-20 class was injected intravenously into each rabbit studied with this class of lipoproteins.

The above procedure was repeated several times, and data were obtained from: 9 rabbits injected with the S_f 20-100 class, 6 rabbits injected with the S_f 15-20 class, and 6 rabbits injected with the S_f 5-15 class. 23 cholesterol fed rabbits were exsanguinated to obtain these lipoproteins. The rabbits injected with these lipoproteins were bled at variable times after the injection, and the serum samples so obtained were analyzed in the ultracentrifuge for lipoprotein distribution.

Alloxan diabetic rabbits fed cholesterol were used as a source of lipoproteins of the S_f 100-400 and S_f 400 and greater classes. As mentioned above, cholesterol fed alloxan diabetic rabbits develop few lipoproteins below S_f 100 and their serum lipids are carried in the enormous quantities of lipoproteins above S_f 100 which they develop. 21 normal rabbits were injected intravenously with five percent alloxan monohydrate solution at a dose of 200 mg/kg. One week after this injection, cholesterol feeding, as described above, was started. After six weeks of cholesterol feeding, there were eight survivors of which three maintained elevated blood sugar levels and developed extreme lipemia. 180 cc of serum were obtained from these three rabbits and was centrifuged at 15,000 rpm for 30 minutes using the Spinco Model L preparative ultracentrifuge. At the end of this time, approximately one cc of a semi-gelled very turbid material floated to the top. This material represents lipoproteins of S_f 400 and greater, and was separated from the balance of the serum by drawing off the subnatant material using a long capillary pipette. The subnatant material was saved for later isolation of the S_f 100-400 class of lipoproteins. Since the S_f 400 and greater class of lipoproteins still contained contaminating lipoproteins of S_f less than 400, the top cc were pooled and resuspended in isotonic saline. Centrifugation was again performed on this material at 15,000 rpm for 30 minutes as above. The subnatant material was discarded, and in this way an effective diluting out of lipoproteins of S_f rate less than 400 was achieved. Two additional ultracentrifugal "washings" were performed, and the final material, constituting a reasonably pure solution of lipoproteins of S_f 400 and greater, was dissolved in 125 cc isotonic saline. 25 cc of this preparation was injected

intravenously into each of five normal rabbits. These rabbits were bled serially for a period up to 14 days after the injections, and the serum was analyzed for lipoproteins in the analytical ultracentrifuge.

The subnatant material from the initial isolation of S_f 400 and greater class of lipoproteins was used as the source of the S_f 100-400 class of lipoproteins. This material was centrifuged at 30,000 rpm for one hour. At the end of this time, very turbid material had floated to the top cc. The subnatant solution was removed by a long capillary pipette and discarded. The lipoproteins in the top cc constitute those whose S_f rate is 100 or greater; since lipoproteins above S_f 400 had already been removed, this fraction contained only lipoproteins of the S_f 100-400 class. The lipoproteins of the S_f 100-400 class were contaminated with lipoproteins of lower S_f classes, and these were removed by adding isotonic saline and recentrifuging and re-isolating the S_f 100-400 class. Three "washings" were performed to obtain reasonably pure lipoproteins of this class. The final purified S_f 100-400 class of lipoproteins was dissolved in 150 cc isotonic saline, and 30 cc was injected intravenously into each of five normal rabbits. Blood specimens were drawn serially up to 14 days after the injections and analyzed for lipoproteins in the analytical ultracentrifuge.

Results

In the initial experiments, the isolated lipoprotein classes were stored in the refrigerator before use. It was soon discovered that these fractions became toxic on standing and had to be used within approximately three days after isolation. Fractions stored longer caused a rapid collapse of the recipient rabbit and death in three to six hours. No apparent couse for this toxicity could be determined. Rabbits which died a few hours after lipoprotein injection are not included in the results. Several other animals were lost as a result of an epidemic of diarrhea. Since diarrhea in the rabbit causes a sharp increase in serum lipoproteins, these animals were not included in the results. No causal relationship between lipoprotein injection and diarrhea could be made, however. As a result of these

exclusions, data is presented on: 4 rabbits receiving injections of lipoproteins of S_f 400 +, 4 receiving lipoproteins of S_f 100-400, 9 receiving S_f 20-100, 6 receiving S_f 15-20, and 6 receiving S_f 5-15. Blood specimens were taken at varying intervals up to a maximum of 14 days after injection. In all cases control blood specimens were obtained, and in most cases specimens were obtained within five minutes of the injection. The 5-minute samples show the nature of the lipoproteins injected which are simply superimposed on the trivial quantities of normally occurring lipoproteins. The concentration of the injected lipoproteins is the result of the initial concentration injected as diluted by the blood volume.

All ultracentrifugal films were analyzed for the S_f 5-15, 15-30, 30-100, and 100-400 classes of lipoproteins. Recent corrections introduced by Gofman et al.[25] were not used because of the magnitude of the observed changes. The measurements of lipoproteins are in these readings directly proportional to the area between an appropriate salt baseline and the pattern representing the lipoproteins.

The data are presented in Figs. 5, 6, and 7 and in Table VI. As can be seen from the data, there is serial conversion with time of all lipoprotein classes above the S_f 5-15 class to the S_f 5-15 class itself. This conversion occurs in a matter of hours and is complete in a few days. The maximal rate of change occurs at 18-24 hours after the injection of lipoproteins above S_f 15. There is considerable variability in the rate of conversion of lipoproteins of higher S_f rate to those of lower S_f rate, however. In addition to the expected variation from rabbit to rabbit, the quantity of lipoprotein injected bears an inverse relation to the rapidity with which it is cleared from the serum and converted to lipoproteins of lower S_f rate. Since variable quantities of lipoproteins of any given class were injected, there is an expected variation in the rate of clearing from one rabbit to another.

Lipoproteins in the S_f 15-20 class were never concentrated to the extent of other higher lipoprotein classes and consequently appear to clear faster than the higher lipoprotein classes.

Table VI

Lipoprotein levels in rabbits injected intravenously with previously isolated classes of lipoproteins and studied at various times after the injection. Control values represent the lipoproteins initially present in the rabbit. All lipoproteins are in mg %.

Injected with lipoproteins of the S_f 400 + class

Rabbit	Time	S_f 5-15	S_f 15-30	S_f 30-100	S_f 100-400	Rabbit	Time	S_f 5-15	S_f 15-30	S_f 30-100	S_f 100-400
	Control	66	7	2	2		Control	24	7	26	9
No. 1	5'	51	0	7	48	No. 4	5'	11	7	7	59
	1-1/2 hr.	44	11	33	114		45'	22	7	55	95
	6 hr.	46	40	112	95		3 hr.	11	15	81	101
	30 hr.	286	51	13	0		18 hr.	53	90	114	18
	4 da.	165	99	17	0		48 hr.	150	48	42	0
	6 da.	207	31	11	0		4 da.	animal found dead			
	11 da.	209	39	47	3						

Injected with lipoproteins of the S_f 100-400 class

Rabbit	Time	S_f 5-15	S_f 15-30	S_f 30-100	S_f 100-400	Rabbit	Time	S_f 5-15	S_f 15-30	S_f 30-100	S_f 100-400
	Control	24	0	0	0		Control	7	2	0	0
No. 6	5'	29	11	62	657	No. 7	5'	37	44	136	1971
	1-1/2 hr.	31	35	180	1146		1-1/2 hr.	15	22	114	1010
	6 hr.	48	48	132	257		6 hr.	24	15	75	183
	30 hr.	143	180	92	9		30 hr.	114	139	209	46
	5 da.	183	29	20	0		5 da.	169	90	40	0
	7 da.	92	55	55	0		7 da.	213	48	29	4
	14 da.	88	48	88	4		14 da.	139	24	7	4

Table VI
(Continued)

Injected with lipoproteins of the S_f 20-100 class

Rabbit	Time	S_f 5-15	S_f 15-30	S_f 30-100	S_f 100-400	Rabbit	Time	S_f 5-15	S_f 15-30	S_f 30-100	S_f 100-400
	Control	11	0	0	0		Control	22	25	39	19
No. X1	5'	29	184	624	106	No. X8	45'	22	251	933	374
	1-1/2 hr.	18	117	253	0		3 hr.	46	290	436	161
	3 hr.	29	88	128	4		14 hr.	178	255	95	4
	6 hr.	48	121	114	0		24 hr.	359	246	110	2
	18 hr.	106	81	55	0		48 hr.	315	290	246	40
	30 hr.	139	15	22	0		3 da.	339	233	244	35
	48 hr.	86	20	9	2		5 da.	103	29	112	59
	3 da.	68	13	29	2		7 da.	88	37	128	106
	5 da.	42	4	0	0						

Rabbit	Time	S_f 5-15	S_f 15-30	S_f 30-100	S_f 100-400	Rabbit	Time	S_f 5-15	S_f 15-30	S_f 30-100	S_f 100-400
	Control	84	31	48	7		Control	30	0	0	0
No. X9	45'	121	363	581	180	No. 21	5'	44	224	354	68
	3 hr.	90	200	392	97		3 hr.	47	168	195	3
	14 hr.	242	229	130	7		18 hr.	196	77	40	0
	24 hr.	323	211	134	4		48 hr.	88	2	40	0
	48 hr.	297	240	172	26		5 da.	53	7	11	0
	3 da.	374	163	123	15		9 da.	167	66	88	9
	5 da.	205	66	33	0		14 da.	70	4	0	0
	7 da.	77	13	4	0						

Table VI
(Continued)

Injected with lipoproteins of the S_f 20-100 class (Continued)

Rabbit	Time	S_f 5-15	S_f 15-30	S_f 30-100	S_f 100-400	Rabbit	Time	S_f 5-15	S_f 15-30	S_f 30-100	S_f 100-400
	Control	84	18	4	0		Control	44	15	7	0
No. 31	5'	118	602	415	129	No. 63	5'	88	268	730	297
	6 hr.	80	171	380	94		3 hr.	88	187	356	26
	30 hr.	167	354	552	57		6 hr.	114	172	209	11
	3 da.	310	455	365	44		18 hr.	113	184	231	69
	8 da.	284	77	40	2		30 hr.	143	102	132	36
	11 da.	132	37	24	0		48 hr.	69	44	36	8
	15 da.	75	48	106	31		3 da.	18	22	29	18

Injected with lipoproteins of the S_f 15-20 class

Rabbit	Time	S_f 5-15	S_f 15-30	S_f 30-100	S_f 100-400	Rabbit	Time	S_f 5-15	S_f 15-30	S_f 30-100	S_f 100-400
	Control	63	25	30	0		Control	44	0	0	0
No. XII	45'	66	194	68	11	No. 22	5'	70	207	35	0
	3 hr.	0	55	44	0		3 hr.	86	81	4	0
	14 hr.	121	40	22	0		18 hr.	169	48	40	4
	24 hr.	174	66	37	4		48 hr.	125	51	24	2
	48 hr.	99	70	70	2		5 da.	70	40	48	26
	3 da.	77	66	165	20						
	5 da.	53	46	51	13						
	7 da.	77	7	9	0						

Table VI
(Continued)

Injected with lipoproteins of the S_f 15-20 class (Continued)

Rabbit	Time	S_f 5-15	S_f 15-30	S_f 30-100	S_f 100-400	Rabbit	Time	S_f 5-15	S_f 15-30	S_f 30-100	S_f 100-400
	Control	44	2	7	4		Control	4	0	0	0
No. 28	5'	180	304	29	0	No. 64	5'	15	139	22	0
	6 hr.	150	62	20	0		3 hr.	29	37	11	0
	30 hr.	154	46	62	7		6 hr.	18	7	0	0
	3 da.	119	26	24	2		18 hr.	11	11	6	0
	8 da.	198	42	33	2		30 hr.	15	26	22	4
	11 da.	95	51	95	11		48 hr.	19	6	0	0
	15 da.	161	31	26	0		3 da.	18	0	0	0
							4 da.	33	19	14	6

Injected with lipoproteins of the S_f 5-15 class

Rabbit	Time	S_f 5-15	S_f 15-30	S_f 30-100	S_f 100-400	Rabbit	Time	S_f 5-15	S_f 15-30	S_f 30-100	S_f 100-400
	Control	168	25	16	0		Control	128	35	53	4
No. X12	45'	264	63	6	0	No. X13	45'	229	93	48	0
	3 hr.	209	47	16	3		3 hr.	183	73	53	7
	14 hr.	341	68	26	0		14 hr.	255	66	53	2
	24 hr.	383	77	37	0		24 hr.	253	103	97	7
	48 hr.	370	95	53	2		48 hr.	288	103	154	26
	3 da.	396	128	99	9		3 da.	112	70	180	55
	5 da.	141	53	55	7		5 da.	68	51	145	31
	7 da.	128	20	9	0		7 da.	99	53	112	7

Table VI
(Continued)

Injected with lipoproteins of the S_f 5-15 class (Continued)

Rabbit	Time	S_f 5-15	S_f 15-30	S_f 30-100	S_f 100-400	Rabbit	Time	S_f 5-15	S_f 15-30	S_f 30-100	S_f 100-400
	Control	33	0	0	0		Control	15	0	0	0
No. 25	5'	361	53	7	9	No. 27	5'	378	66	20	11
	3 hr.	271	22	4	0		6 hr.	198	11	0	0
	18 hr.	240	26	15	2		30 hr.	117	26	7	0
	48 hr.	90	15	95	26		3 da.	130	84	46	2
	5 da.	64	42	92	22		8 da.	51	53	64	2
	9 da.	59	20	37	4		11 da.	48	35	84	9
							15 da.	42	40	70	4

The higher the S_f rate of the lipoproteins injected, the more time is required to convert the injected lipoproteins to the S_f 5-15 class. Conversion begins rapidly and is apparent within 3 hours; however, the time required to clear the serum of, e.g., the S_f 100-400 class of lipoproteins and convert to the S_f 5-15 class is greater than the time required to clear the serum of, e.g., the S_f 20-100 class of lipoproteins and convert these to the S_f 5-15 class.

As can be seen from the data, there is an invariable direction of change of lipoproteins after injection. All lipoproteins injected are converted successively to lower classes of lipoproteins. After the injection of the highest class of lipoproteins used in this study, the S_f 400 and greater class, there is a transient elevation of S_f 100-400 class with disappearance of the S_f 400 and greater class. Next the S_f 100-400 class diminishes and the S_f 20-100 class appears. This process continues until only the S_f 5-15 class is elevated, and with time this class of lipoproteins shrinks to normal size. Similarly any injected class of lipoproteins above S_f 15 disappears with the appearance of lower classes of lipoproteins. Every lower class of lipoproteins is transiently elevated as the conversion process continues through the lower S_f classes, and no class of lipoproteins fails to participate in this serial change. This change occurs only in one direction. When large quantities of the S_f 5-15 class are injected, no lipoproteins of higher S_f class appear; the S_f 5-15 class merely shrinks with time. Similarly after the injection of, e.g., the S_f 20-100 class of lipoproteins, no increase in the S_f 100-400 class is ever seen. The clearance of the S_f 5-15 class of lipoproteins is a slower process than the conversion of higher classes of lipoproteins to lower classes, the former requiring days and the latter occurring in hours.

The quantities of lipoproteins injected show a progressive loss of concentration as the lipoproteins convert to lower classes (Fig. 5). After the injection of the S_f 100-400 class, there appears a quantity of the S_f 30-100 class which is considerably less than the amount of S_f 100-400 which was injected. As the S_f 30-100 class

converts to the S_f 15-30 class, a still lesser amount of material appears in this class. Finally, the lipoproteins which appear in the S_f 5-15 class show the least quantitative increase of any class of lipoproteins. There appears to be a progressive loss of lipids from the serum paralleling the conversion of lipoproteins of high S_f rate to those of lower S_f rate.

The term conversion used in describing the above changes implies nothing as to the mechanism involved. It is conceivable there is a progressive loss of fragments of the lipoprotein molecule in this process with the same basic structure remaining. It is also possible that lipoproteins of high S_f rate are cleared from the serum followed by synthesis of lipoproteins of lower S_f rate.

The removal of large quantities of blood from the rabbit may lead to an increase in serum lipoproteins in 2-7 days as mentioned above. On the average, one out of three rabbits of the strain used at this laboratory shows this response to repeated hemorrhage. Many of the rabbits reported in this study were bled in sufficient quantities to render them liable to an increase in lipoproteins from bleeding alone. The rise in lipoproteins observed in rabbits X8 and X11 in the S_f 30-100 and S_f 100-400 classes which occurred after 48 hours may be attributed to this effect. Undoubtedly the effect of bleeding alone on lipoprotein changes serves to obscure somewhat the results reported above and in several cases interferes with the steady decline of each lipoprotein class after injection. The overall direction of change is nevertheless clear in every animal studied.

The ultracentrifugal isolation of the various classes of lipoproteins injected was not complete, and in many instances lipoproteins of classes other than those stated to be injected were present. This explains the appearance of, e.g., the S_f 100-400 class five minutes after the injection of the so-called S_f 20-100 class. Quantitative isolation of any class of lipoproteins completely free of all other classes of lipoproteins would yield much smaller quantities of lipoproteins than those injected here. The lipoproteins injected in this study were predominantly of one class but were contaminated with small amounts of the adjacent classes of lipoproteins. Huge quantities

of lipoproteins could be isolated to this degree of purity. Without loading the normal rabbit to this degree, the above changes occur too rapidly and with too low a concentration of converted lipoproteins to be clearly seen.

One rabbit, No. 6, showed an increase in lipoproteins of the injected class at 1-1/2 hours after the injection as compared with five minutes after the injection. The only explanation for this observation is that incomplete mixing with the blood had occurred. The five minute sample was drawn as soon as possible after the injection, and it is possible that the true time of sample collection was shorter than the stated five minutes.

Discussion

The above data indicate clearly that a stepwise conversion of lipoproteins occurs in the rabbit and that this conversion is always in a direction from high S_f rate to low S_f rate. In general, the higher the S_f rate of the lipoprotein, the faster does it convert to lower S_f classes. An injection of the normally occurring S_f 5-15 class is followed by a steady reduction in this class of lipoproteins, and no lipoproteins of higher S_f rate appear. The clearance from the serum of the S_f 5-15 class is much slower than the clearance of the higher S_f classes and takes several days to become complete. The clearance of all lipoprotein classes above S_f 15 occurs in a matter of hours, and they are rapidly converted to lipoproteins of lower S_f rate. Nichols et al.[30] have studied the lipoproteins in rabbit and rat lymph obtained from the thoracic duct and find lipoproteins predominate whose S_f rate is 100 or greater. It seems reasonable from this observation to conclude that fats absorbed by the intestine are represented by lipoproteins whose S_f rate is greater than 100 and that these lipoproteins are delivered to the blood as such. The conversion process described in this paper may represent the next stage in fat transport as lipoproteins are converted from S_f 100 and greater to the normally occurring S_f 5-15 class. It is of interest in this connection that the actual quantity of lipoproteins delivered as

S_f 100 and greater eventually produces a rise in lipoproteins in the S_f 5-15 class, which is much smaller in quantity. The difference may reflect the fats stored or metabolized by the animal. The concept of cholesterol, phospholipid, and protein acting as prosthetic groups in lipoproteins for the transport of fat may be mentioned. Since the neutral fat content of lipoproteins rises rapidly with increasing S_f rate, neutral fat would seem to be the principal component lost with the conversion of lipoproteins from high S_f rate to lower S_f rate. The presence of an elevated S_f 12-100 class of lipoproteins represents a situation where the conversion process is incomplete or unable to keep up with the influx of lipoproteins of high S_f rate. This is of interest in view of the association of the S_f 12-100 classes of lipoproteins and atherosclerosis and suggests a link between atherosclerosis and impaired fat transport.

The clearance of lipoproteins after injection is directly influenced by the quantity injected, and huge quantities are cleared more slowly than smaller amounts. This undoubtedly indicates that there is a limit to the quantity of lipoproteins of high S_f rate which can be converted to lipoproteins of lower S_f rate in a given interval of time. The rapidity with which the animal can clear lipoproteins of the S_f 5-15 class is much slower than the rapidity with which it clears lipoproteins of higher S_f class. The metabolic fate of the S_f 5-15 class of lipoproteins may well be different than that of lipoproteins of S_f greater than 15. This is suggested by the continuous presence of the S_f 5-15 class of lipoproteins in normal animals, as well as the different clearing rate of this class after injection.

The data reported in this paper indicate the great difficulty of attempting to produce atherosclerosis in the rabbit by direct injection of various lipoprotein classes. Tremendous quantities of any lipoproteins above S_f 15 would have to be continuously injected, because of the rapid conversion of these lipoproteins to the S_f 5-15 class. Furthermore, the injection of any class of lipoproteins would produce an elevation of all lipoprotein classes of lower S_f rate as conversion from high S_f to lower S_f occurs.

These findings corroborate and extend the observations made on the conversion of lipoproteins during recovery from agents which cause an elevation of serum lipoproteins. Gofman has reported studies on humans followed after a high fat meal. Here again, lipoproteins of high S_f rate appear in the serum after eating and then convert to lipoproteins of lower S_f rate with progressive loss of concentration as the conversion proceeds. Similarly, heparin injected into humans or rabbits causes a conversion of lipoproteins from high to lower S_f rate.[31]

In view of the ability of heparin to instigate conversion of lipoproteins from high to lower S_f rate, further observations were made as to the possible role of heparin in the lipoprotein conversion reported above. While heparin in vitro is inactive, an injection of heparin into an animal causes the appearance of an "active factor" in the serum which does convert lipoproteins in vitro. Blood specimens were obtained from rabbits which had been injected with lipoproteins of the S_f 20-100 class. The blood specimens were obtained at various times after the injection of the lipoproteins and during the period when lipoprotein conversion was occurring at its fastest rate. These blood specimens were then analyzed for the presence of "active factor" using the egg lipoprotein test of Nichols[32] et al. This test depends on the ability of "active factor" containing serum to clear a turbid preparation of egg yolk lipoprotein. At no time was any evidence for active factor found even though the rate of lipoprotein conversion was proceeding at a rate much greater than that seen after heparin injection in the cholesterol fed rabbit. It is entirely possible that the test used was inadequate or not sufficiently sensitive to detect any "active factor" activity. Experiments are under way to examine the possibility of an acceleration of lipoprotein conversion with the simultaneous injection of heparin and isolated lipoprotein classes.

The role of endogenous heparin in the various experimental regimens which increase lipoproteins in the rabbit is obscure at the present. The rabbit is known to be an animal with relatively few mast cells[33] and this finding may be related to the ease with which lipoproteinemia can be produced in this species. Cortisone has been

reported to reduce the number of mast cells[34] and this observation may be related to the lipemia produced by this hormone. Perhaps the rabbit with initially fewer mast cells is easily pushed below a critical level of endogenous heparin by a substance like cortisone which exerts deleterious effects on mast cells. There is no evidence as to the status of mast cells in alloxanized rabbits. Undoubtedly many factors are at work influencing the lipoprotein distribution in serum, and heparin may well be only the first clearly defined agent to be identified which has profound effects on lipid metabolism.

Summary

1. Normal and cholesterol fed rabbits were injected with CCl_4 and their serum cholesterol levels and lipoproteins of the S_f 3-12, 12-20, and 20-40 classes measured during and after the injections. CCl_4 produced a marked increase above control levels in all classes of lipoproteins and in cholesterol in the non-cholesterol fed rabbit. These substances gradually decreased to control levels after the cessation of CCl_4 injections. No macroscopic atherosclerosis developed in this non-cholesterol fed group of animals as was predicted by the relatively low concentration of the S_f 12-40 class of molecules present for this limited amount of time. In the cholesterol fed rabbit, cholesterol and all classes of lipoproteins increased during CCl_4 injections but continued to increase after the cessation of CCl_4 injections. Very large quantities of serum lipoproteins and cholesterol developed in the cholesterol fed rabbits by the end of ten weeks, and at this time all animals had developed atherosclerosis.

The data suggest that the increase in the normally occurring S_f 3-12 class and the appearance of high concentrations of S_f 12-20 and 20-40 classes of lipoproteins may occur as a result of impaired function of the degradation and synthetic system (possibly in the liver) involved in the metabolism of these molecules.

2. The blood levels of the S_f 10-20 class of molecules measured in 34 patients with chronic hepatitis showed no significant difference from the level found in clinically normal individuals of corresponding age and sex. These data offer no support for the contention that chronic hepatitis is accompanied by lesser degrees of atherosclerosis than is seen in the general population.

3. The S_f 0-12, 12-20, and 20-100 classes of lipoproteins are significantly elevated in patients with infectious or serum hepatitis when compared with normals of matched age and sex. The S_f 100-400 class of lipoproteins is significantly reduced. The flotation rate of the major peak in the S_f 0-12 class is significantly increased in these patients. Lipoprotein measurements do not segregate infectious from serum hepatitis.

4. There is a significant positive correlation between S_f 0-12, 12-20 and 20-100 classes of lipoproteins and the icterus index. There is a significant negative correlation between the S_f 100-400 class and the icterus index. Similar correlations with serum bilirubin (both direct and total) are found. There is a significant positive correlation between the flotation rate of the major peak in the S_f 0-12 class and icterus index.

No significant correlations between icterus index and lipoproteins are found in patients whose icterus index is 10.4 or less. Lipoproteins of S_f less than 100 are not significantly different in these hepatitis patients when compared with healthy normals.

There is a significant positive correlation between the S_f 12-20 class of lipoproteins and icterus index and a significant negative correlation between the S_f 100-400 class of lipoproteins and icterus index in hepatitis patients whose icterus index is 10.5 or greater. Lipoproteins of the S_f 0-12, 12-20, and 20-100 classes are significantly elevated in hepatitis patients whose icterus index is 10.5 or greater; lipoproteins of the S_f 100-400 class are significantly reduced.

5. There is no significant correlation between any class of lipoproteins and the albumin/globulin ratio, gamma globulin level, zinc turbidity, or cephalin-cholesterol flocculation. The entire S_f 0-400 group of lipoproteins correlates significantly with the thymol turbidity test; no subclass within this group correlates significantly. The S_f 20-100 class of lipoproteins correlates significantly with the total lipid determination of Kunkel, Ahrens and Eisenmenger (phenolic precipitation). The S_f 12-20 class correlates with the colloidal red test. No other class of lipoproteins correlates with these two latter tests. The return of lipoprotein levels to normal during convalescence parallels the return of the icterus index to normal.

6. Normal rabbits were injected with lipoproteins isolated from cholesterol fed rabbits. Lipoproteins of the S_f 5-15, 15-20, 20-100, 100-400, and 400 + classes were studied. Within a few hours, lipoproteins of high S_f rate converted to lipoproteins of lower S_f rate in a serial fashion. Conversion was always from high to low and never in the reverse direction. Injected lipoproteins of the S_f 5-15

class disappeared from the serum and no lipoproteins of higher S_f rate appeared at any time. The disappearance of injected lipoproteins of the S_f 5-15 class proceeded much more slowly than the conversion of lipoproteins of high S_f rate to this class. Conversion of lipoproteins of high S_f rate to lower S_f rate was accompanied by a progressive lowering of concentration in the lower S_f classes. Eg: S_f 100-400 → less S_f 30-100 → still less S_f 15-30, etc.

Acknowledgments

The author is deeply indebted to Dr. John W. Gofman for his many helpful suggestions, continued interest, and useful advice. The guidance and inspiration of Dr. Gofman have been a source of real pleasure during the various phases of this work. The author is also especially grateful to Dr. Hardin B. Jones for his personal interest and his many constructive ideas during the course of this research.

Thanks are due to Dr. John Lawrence for his enthusiastic support and to the entire Donner Laboratory group for making available the necessary equipment and laboratory space. In particular, appreciation is extended to the group studying lipoprotein metabolism for their fine cooperation and interesting exchange of ideas.

Appreciation is also extended to the Atomic Energy Commission, The National Heart Institute of the U. S. Public Health Service, and the Life Insurance Medical Research Fund for their support of this work.

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Figure Captions

Fig. 1 a. Ultracentrifugal photographs showing the flotation of low density lipoproteins of a normal rabbit. Each frame is ruled for calculation of the S_f rate of any peak appearing in that frame. In this and all the other series of photographs, successive frames are at 0, 6, 12, 22, 30, and 38 minutes after the ultracentrifuge rotor has reached full speed (52, 640 rpm.). Consequently, these S_f markings can be used on corresponding frames in all the series of photographs below. In this pattern, the low density lipoproteins from 5 cc of serum were concentrated into 1 cc by preparative ultracentrifugation and then analyzed in the ultracentrifuge as described above.

Fig. 1 b. Flotation pattern of low density lipoproteins of a rabbit after two weeks of carbon tetrachloride injections, showing markedly increased levels of lipoproteins. The low density lipoproteins from 3 cc of serum were concentrated into 1 cc in this pattern. Consequently, the increase in lipoproteins is 67 percent greater than represented by these photographs when compared with the pattern above.

Fig. 1 c. Flotation pattern of low density lipoproteins from a rabbit one week after the cessation of carbon tetrachloride injection. This shows the return toward normal levels of the lipoproteins of S_f 20-40 class, with those lipoproteins of higher S_f value disappearing first. The lipoproteins of 3 cc serum were concentrated into 1 cc in this pattern.

Fig. 1 d. Flotation pattern of low density lipoproteins from a rabbit two weeks after the cessation of carbon tetrachloride injections. The molecules of the S_f 20-40 class are greatly reduced in concentration, and those of the S_f 12-20 class are disappearing with those of higher S_f value disappearing first. The lipoproteins of 5 cc of serum were concentrated into 1 cc in this pattern.

Fig. 1 e. Flotation pattern of low density lipoproteins from a rabbit 4 weeks after the cessation of carbon tetrachloride. Molecules of the S_f 20-40 and 12-20 classes have returned almost to normal levels and the 3-12 class is beginning its return to normal levels. The lipoproteins of 5 cc of serum were concentrated into 1 cc in this pattern.

Fig. 2 Diagram illustrating the levels of S_f 10-20 molecules in patients with cirrhosis and normal individuals of corresponding age and sex.

Fig. 3 Figure 3 illustrates the changes in lipoprotein levels, flotation rates of the major peak, and several liver function tests during the course of viral hepatitis. These graphs represent the mean of all values obtained in the patients studied. The onset of jaundice is taken as Day 1.

Fig. 4 Figure 4 shows the changes in lipoprotein levels, flotation rates of the major peak and several liver function tests in each of four patients studied for a part of the course of viral hepatitis. Day 1 represents the onset of jaundice.

Fig. 4 a. 21 year old male with infectious hepatitis discharged as well on the 9th week of illness.

Fig. 4 b. 19 year old male with infectious hepatitis discharged on the 8th week of illness. Two weeks later he was rehospitalized with a recurrence of symptoms.

Fig. 4 c. 26 year old male with infectious hepatitis discharged as well on the 18th week of illness.

Fig. 4 d. 37 year old male with infectious hepatitis discharged as well on the 16th week of illness.

Fig. 5 Bar graph showing changes in lipoprotein levels of various classes after injection of an isolated class of lipoproteins. The abscissa represents time after injection. The lipoprotein class injected was contaminated with small amounts of adjacent classes of lipoproteins.

Fig. 5 This accounts for the slight rise of classes of lipoproteins adjacent to the class stated to be injected 5 minutes after injection. The absence of shaded area at a particular time indicates 0 mg% present.

Fig. 5 a. Injection of the S_f 5-15 class of lipoproteins. This illustrates the clearance of this class with no lipoproteins of higher S_f rate appearing.

Fig. 5 b. Injection of the S_f 15-20 class of lipoproteins.

Fig. 5 c. Injection of the S_f 20-100 class of lipoproteins.

Fig. 5 d. Injection of the S_f 100-400 class of lipoproteins.

Fig. 5 e. Injection of the S_f 400 + class of lipoproteins. This graph, together with B, C, and D illustrates the conversion of lipoproteins of high S_f rate to those of lower S_f rate with progressive loss of concentration as the conversion proceeds.

Fig. 6 Line graphs showing the changes in serum lipoprotein levels after the injection of isolated lipoprotein classes. The abscissa represents time after injection. As in Fig. 5, the injected lipoprotein class was contaminated with small amounts of adjacent classes of lipoproteins accounting for the slight rise in these classes five minutes after injection. The absence of the lines during certain time intervals or for control levels indicates 0 mg%.

Fig. 6 a. Injection of the S_f 5-15 class of lipoproteins showing clearance of this class with no rise in levels of lipoproteins of higher S_f rate.

Fig. 6 b. Injection of the S_f 15-20 class of lipoproteins.

Fig. 6 c. Injection of the S_f 20-100 class of lipoproteins.

Fig. 6 d. Injection of the S_f 100-400 class of lipoproteins.

Fig. 6 e. Injection of the S_f 400 + class of lipoproteins. This graph, together with B, C, and D shows the serial conversion of lipoproteins of high S_f rate to those of lower S_f rate.

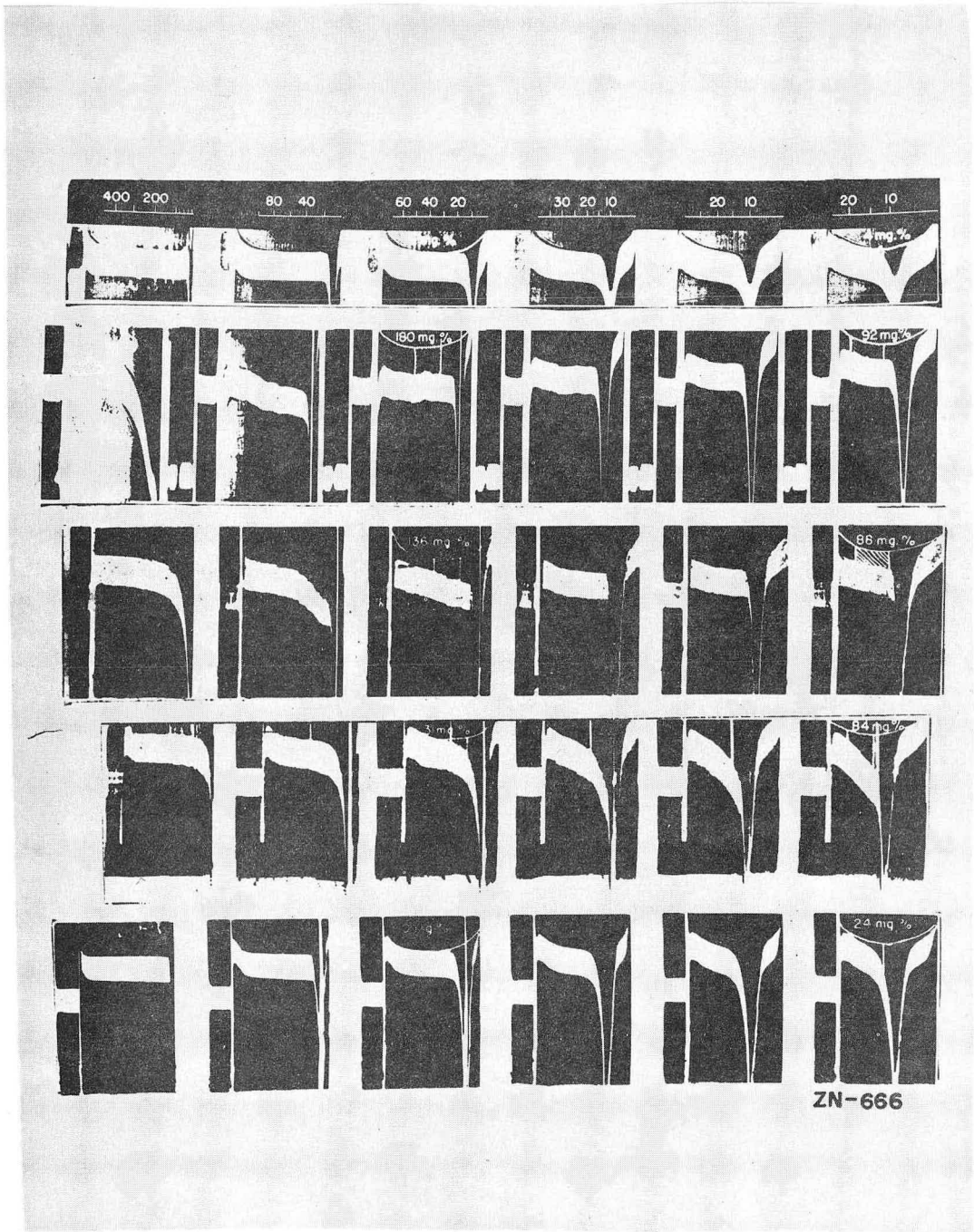
Fig. 7 A series of ultracentrifugal photographs showing the changes in pattern after the injection of the S_f 20-100 class of lipoproteins into a normal rabbit.

Fig. 7 a. Ultracentrifugal photographs showing the control levels of low density lipoproteins in the normal rabbit used in this experiment. (Upper pattern). In this and in all other series of photographs successive frames are at 0, 6, 12, 22, 30 and 38 minutes after the ultracentrifuge rotor has reached full speed (52, 640 revolutions per minute). The low density lipoproteins from 5 cc of serum were concentrated into 1 cc by preparative ultracentrifugation in this and all other series of photographs, and then analyzed in the ultracentrifuge as described above.

Fig. 7 b. Levels of lipoproteins 5 minutes after the injection of the S_f 20-100 class. The lipoproteins injected are simply added to those already present.

Fig. 7 c. Levels of lipoproteins 18 hours after the injection of the S_f 20-100 class. Injected lipoproteins are converting to those of lower S_f rate.

Fig. 7 d. Levels of lipoproteins 72 hours after the injection of the S_f 20-100 class. The injected lipoproteins have been converted to the S_f 5-15 class which still remains elevated as compared to control levels. Each frame in this series is ruled for calculation of the S_f rate of any peak appearing in that frame. These S_f markings can be used on corresponding frames in all series os photographs.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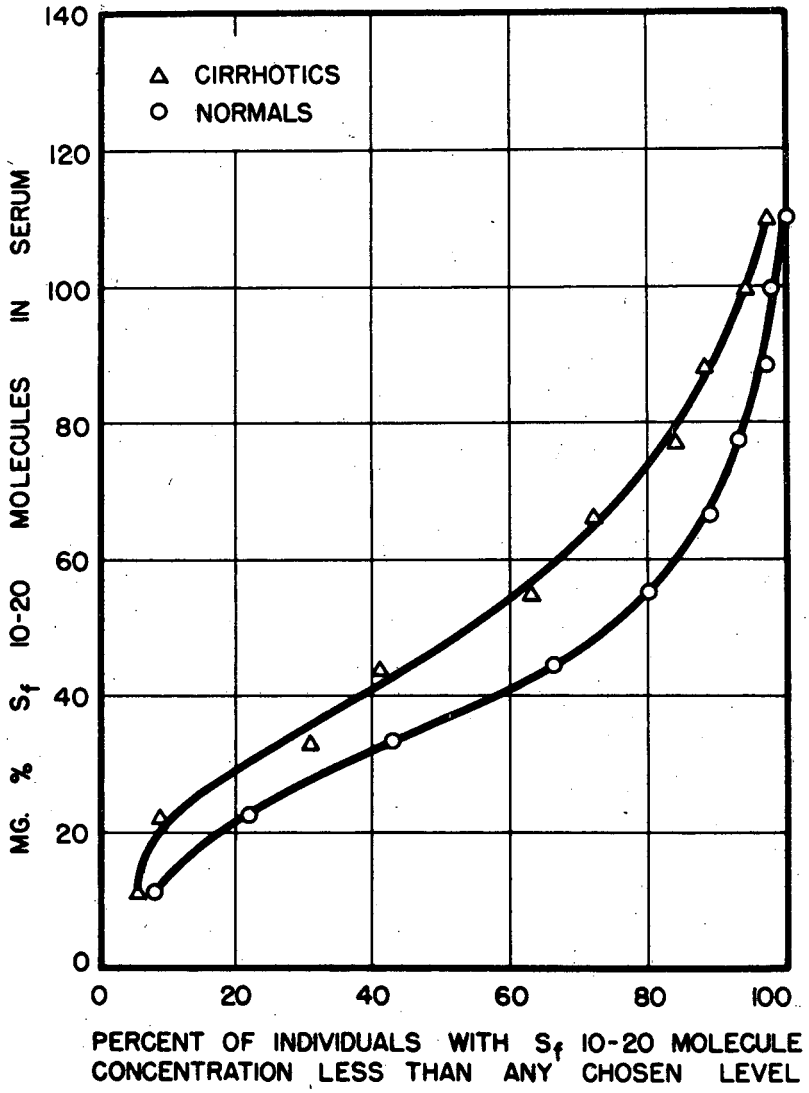
24 mg. %
ZN-666

Fig. 1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MG. % S_f 10-20 MOLECULES IN SERUM
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100
120
140
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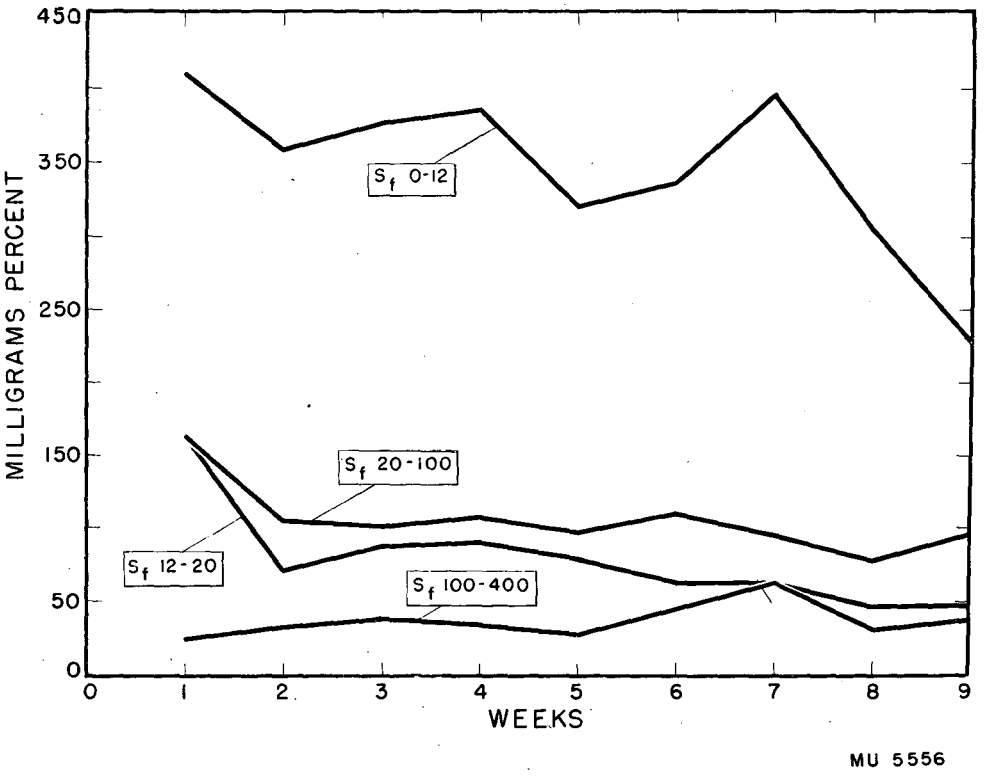
60
80
100
PERCENT OF INDIVIDUALS WITH S_f 10-20 MOLECULE CONCENTRATION LESS THAN ANY CHOSEN LEVEL
MU 2470

Fig. 2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S_f 12-20
S_f 100-400
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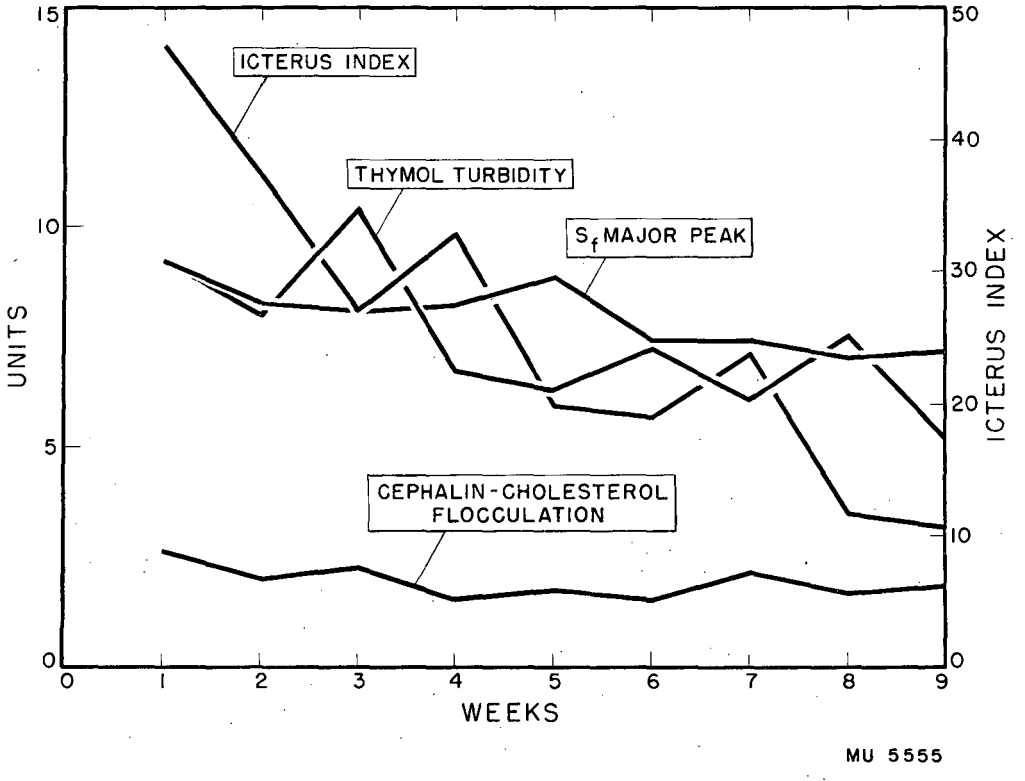
Fig. 3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10
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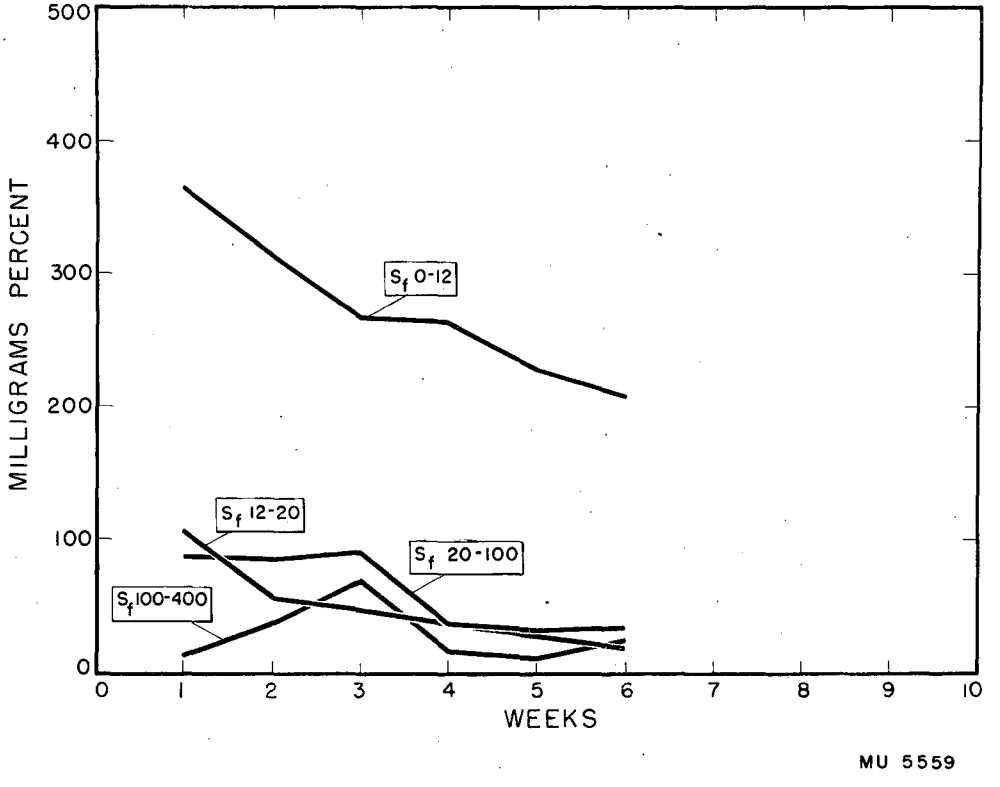
8
9
MU 5555

Fig. 3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S_f 20-100
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4
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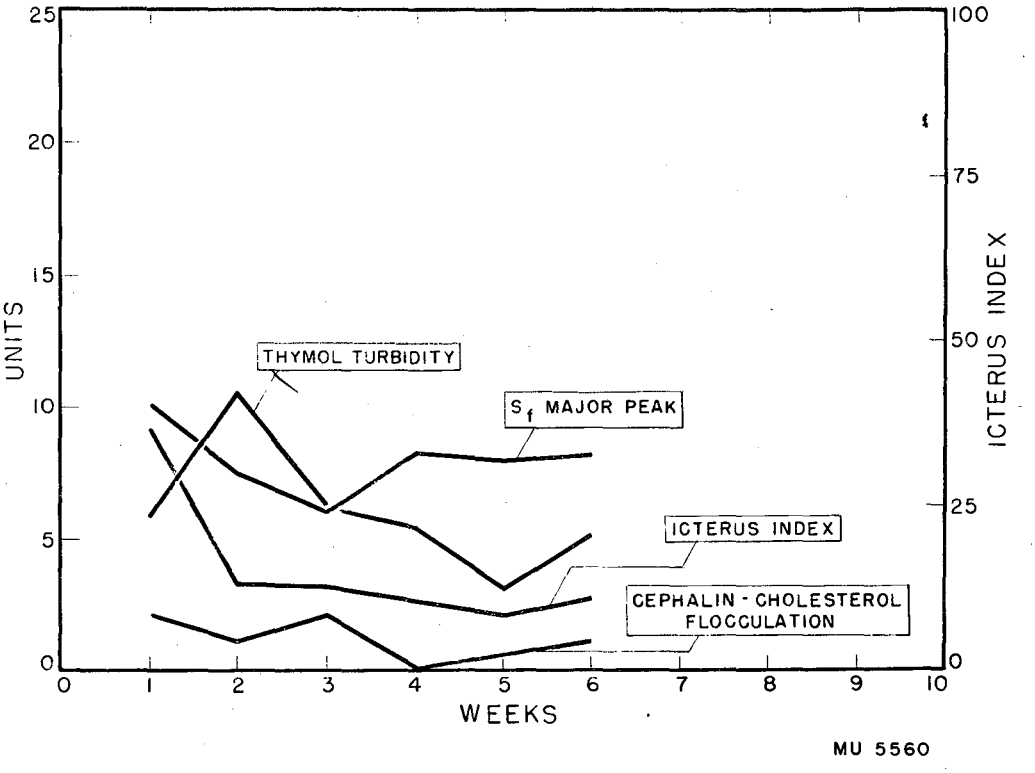
6
7
8
9
10
WEEKS
MU 5559

Fig. 4 A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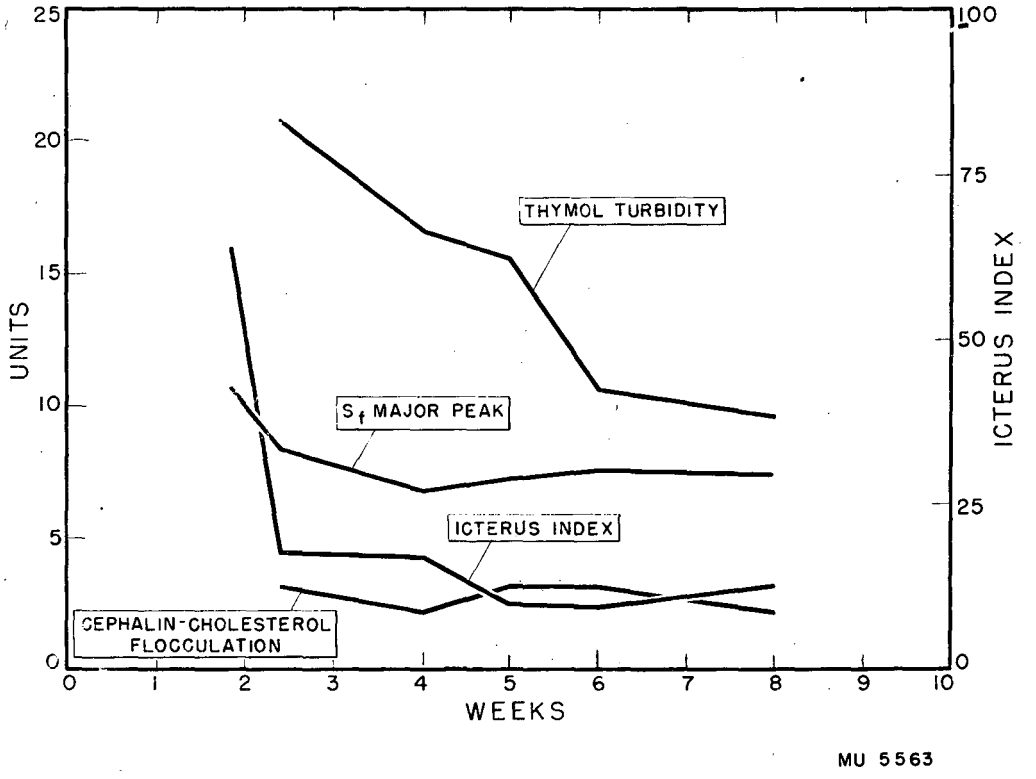
WEEKS
MU 5560

Fig. 4 A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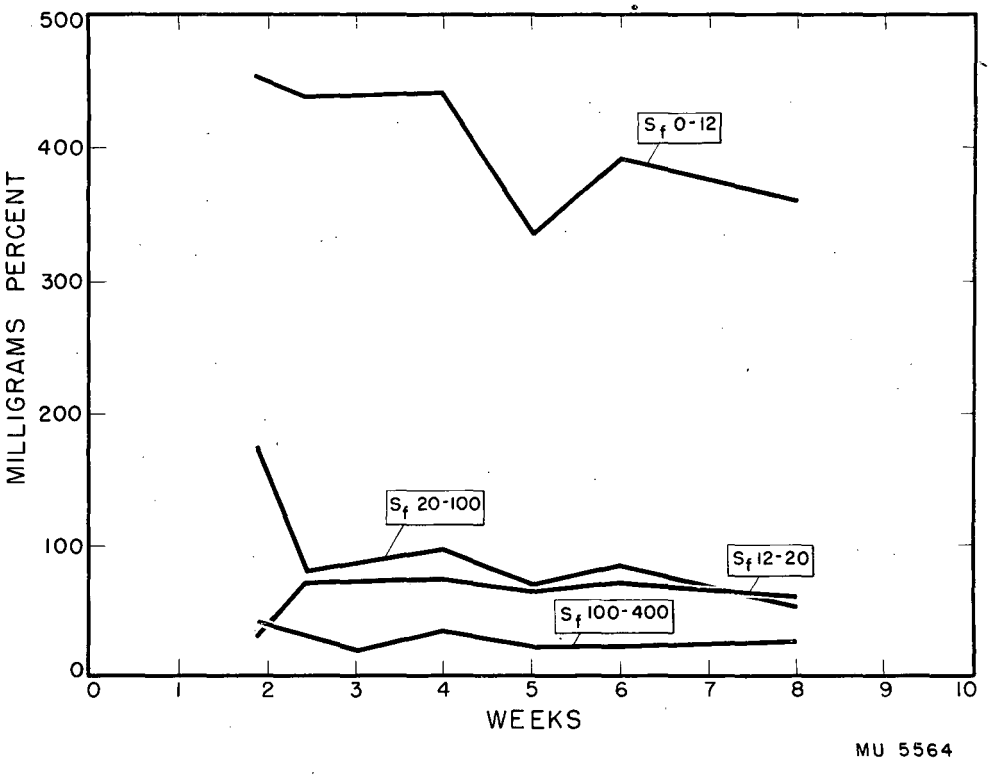
WEEKS
MU 5563

Fig. 4 B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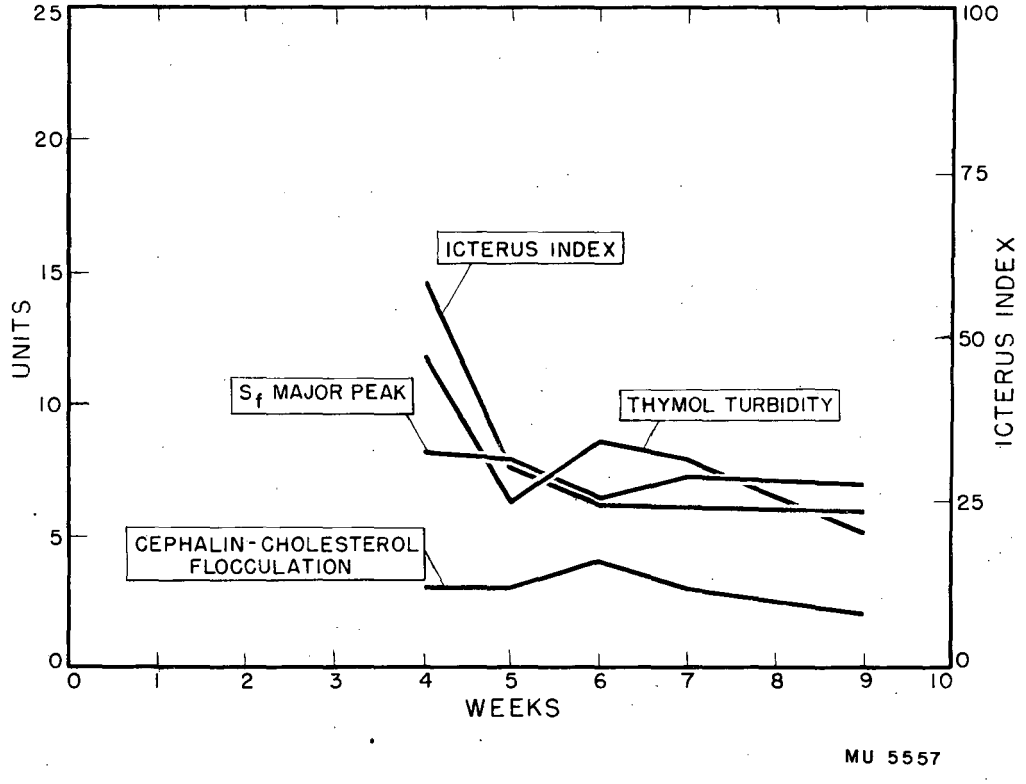
S_f 12-20
S_f 100-400
MU 5564

Fig. 4 B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WEEKS
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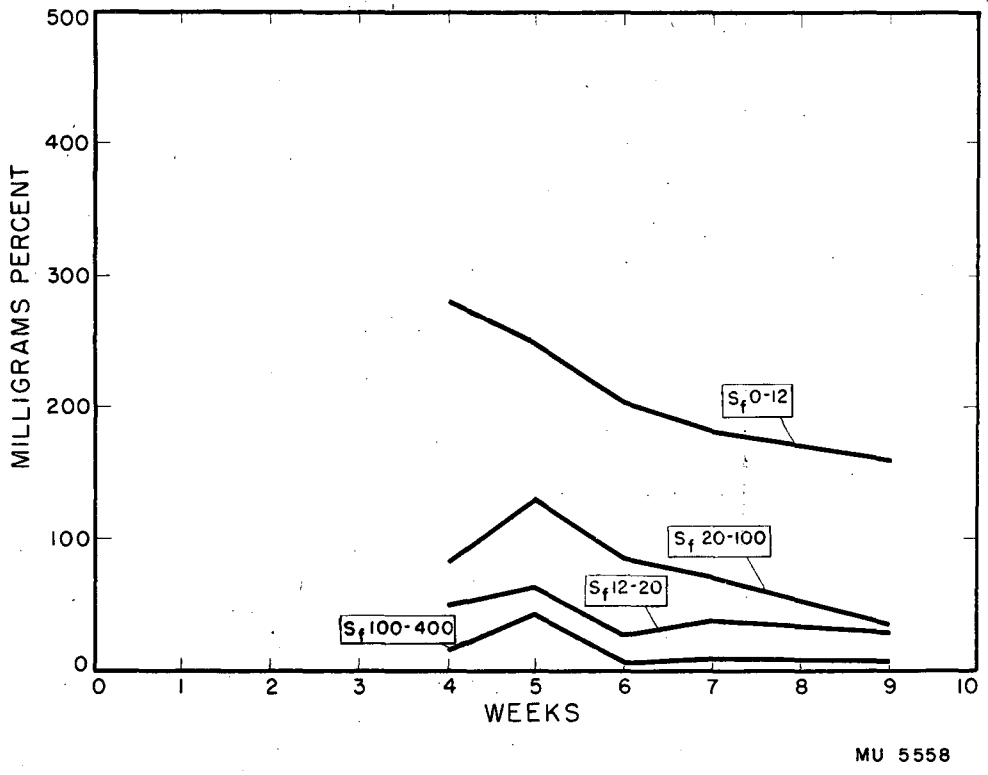
THYMOL TURBIDITY
CEPHALIN-CHOLESTEROL FLOCCULATION
MU 5557

Fig. 4 C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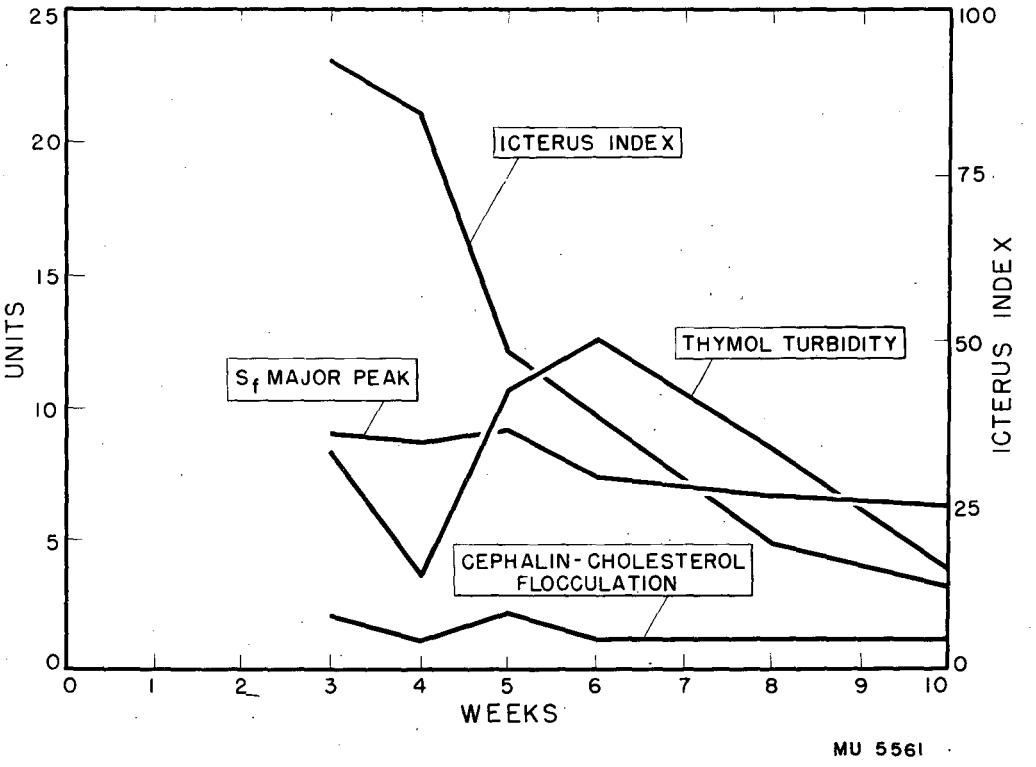
S_f 12-20
S_f 100-400
MU 5558

Fig. 4 C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CEPHALIN-CHOLESTEROL FLOCCULATION
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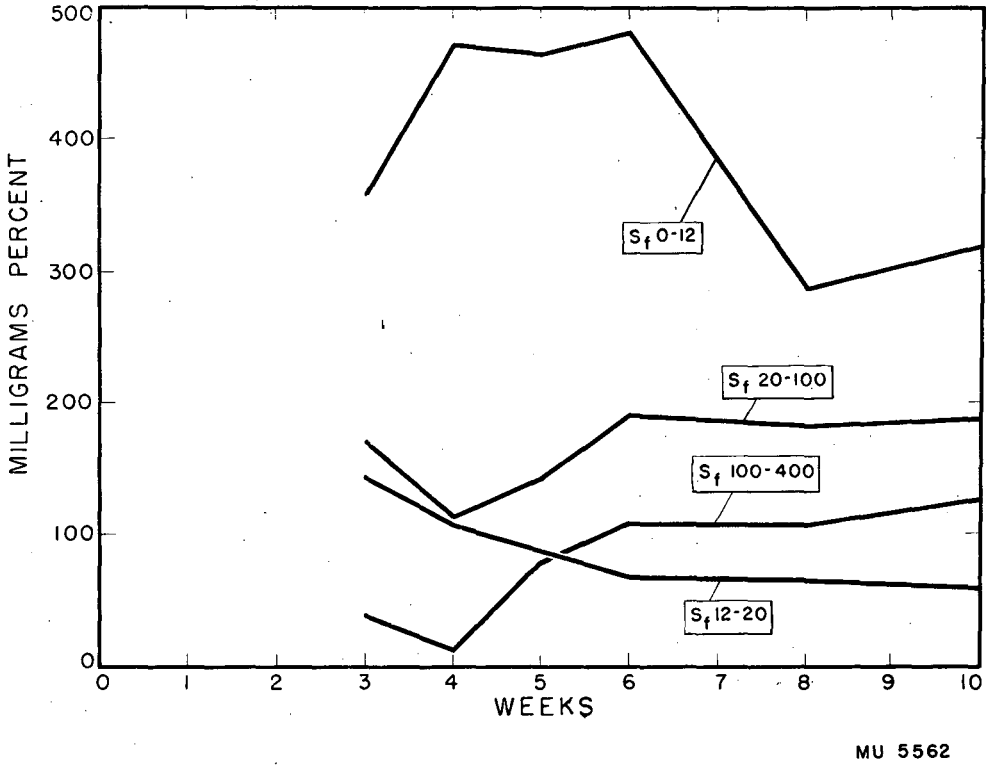
WEEKS
MU 5561

Fig. 4 D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WEEKS
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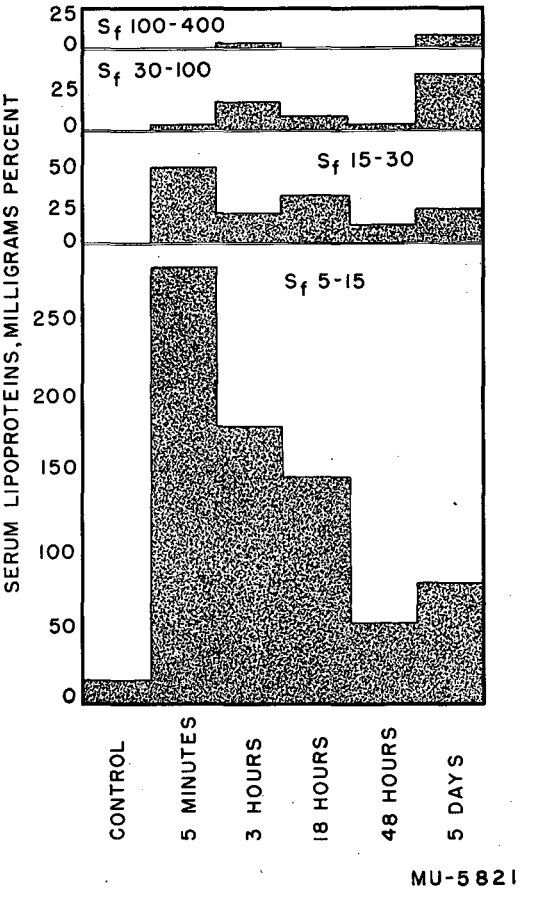
S_f 12-20
MU 5562

Fig. 4 D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S_f 5-15
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25
0
50
25
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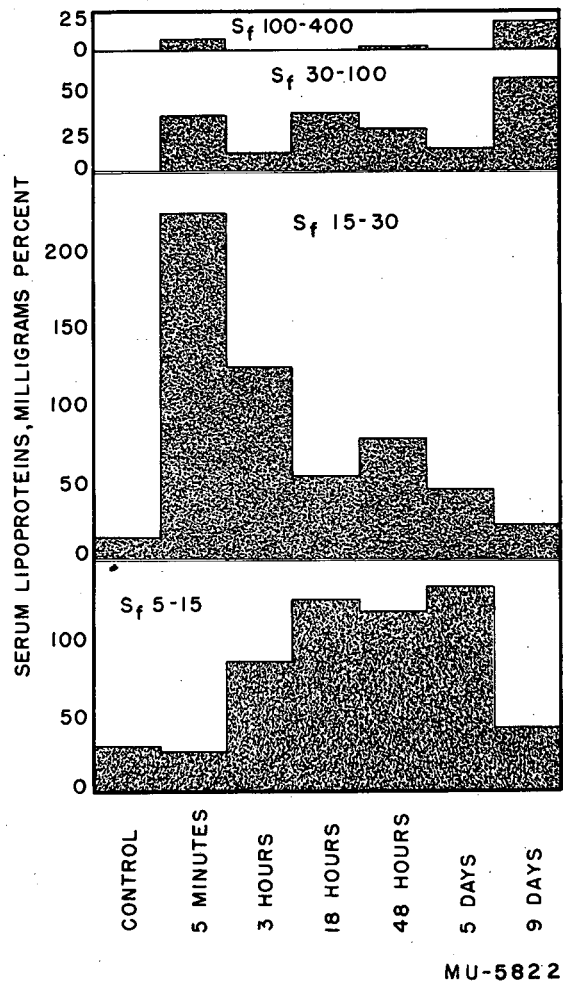
18 HOURS
48 HOURS
5 DAYS
MU-5821

Fig. 5 A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100
50
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SERUM LIPOPROTEINS, MILLIGRAMS PERCENT
CONTROL
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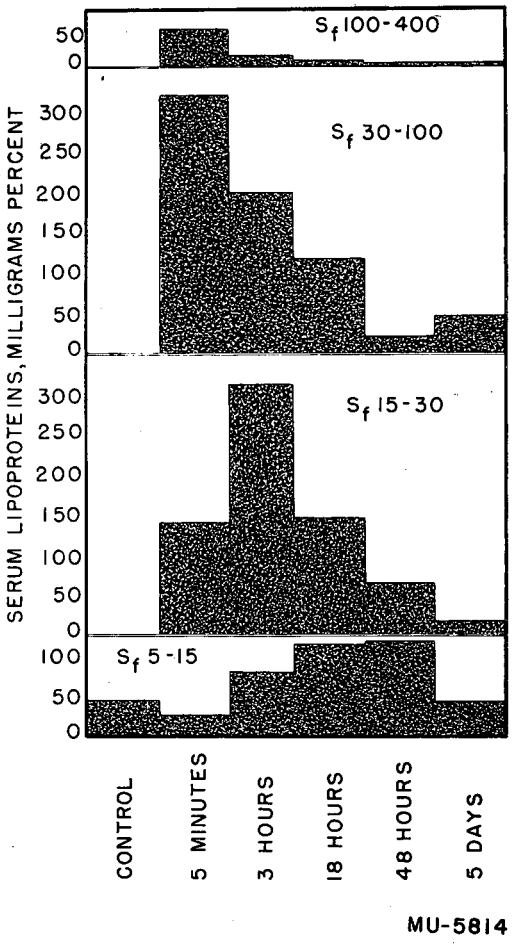
3 HOURS
18 HOURS
48 HOURS
5 DAYS
9 DAYS
MU-5822

Fig. 5 B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200
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CONTROL
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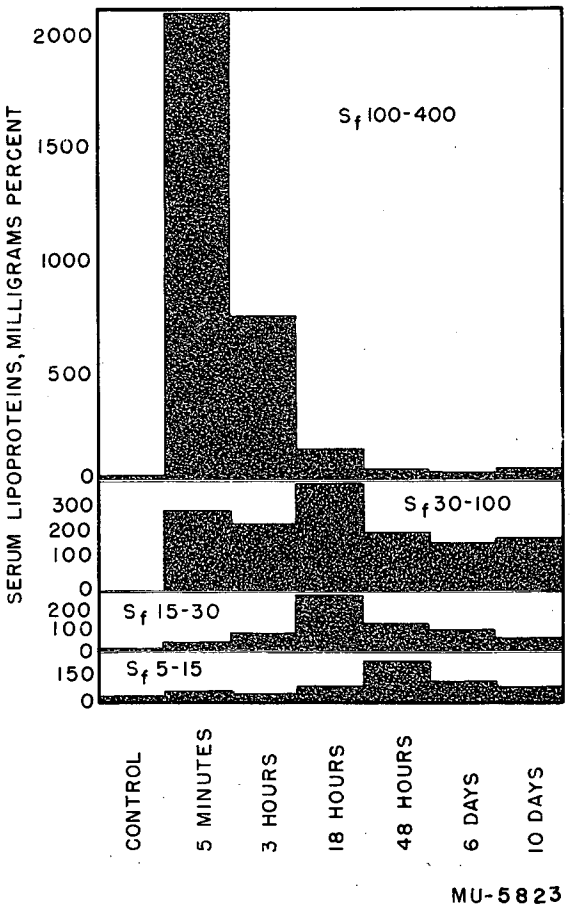
3 HOURS
18 HOURS
48 HOURS
5 DAYS
MU-5814

Fig. 5 C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200
100
0
S_f 15-30
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CONTROL
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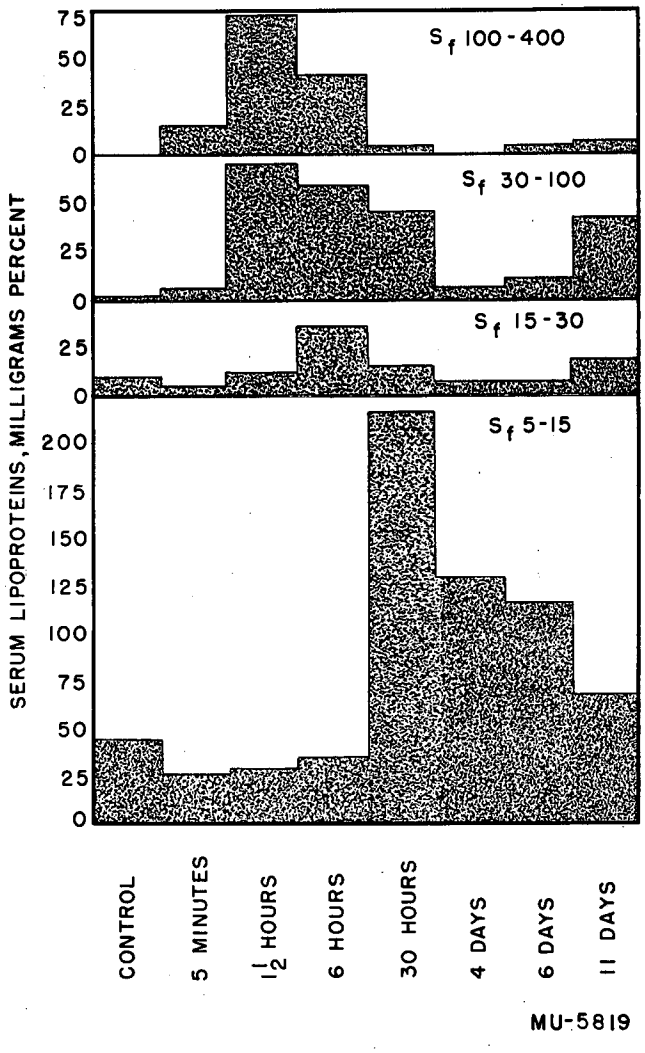
3 HOURS
18 HOURS
48 HOURS
6 DAYS
10 DAYS
MU-5823

Fig. 5 D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200
175
150
125
100
CONTROL
5 MINUTES
1½ HOURS
6 HOURS
30 HOURS
4 DAYS
6 DAYS
11 DAYS
MU-5819

Fig. 5 E

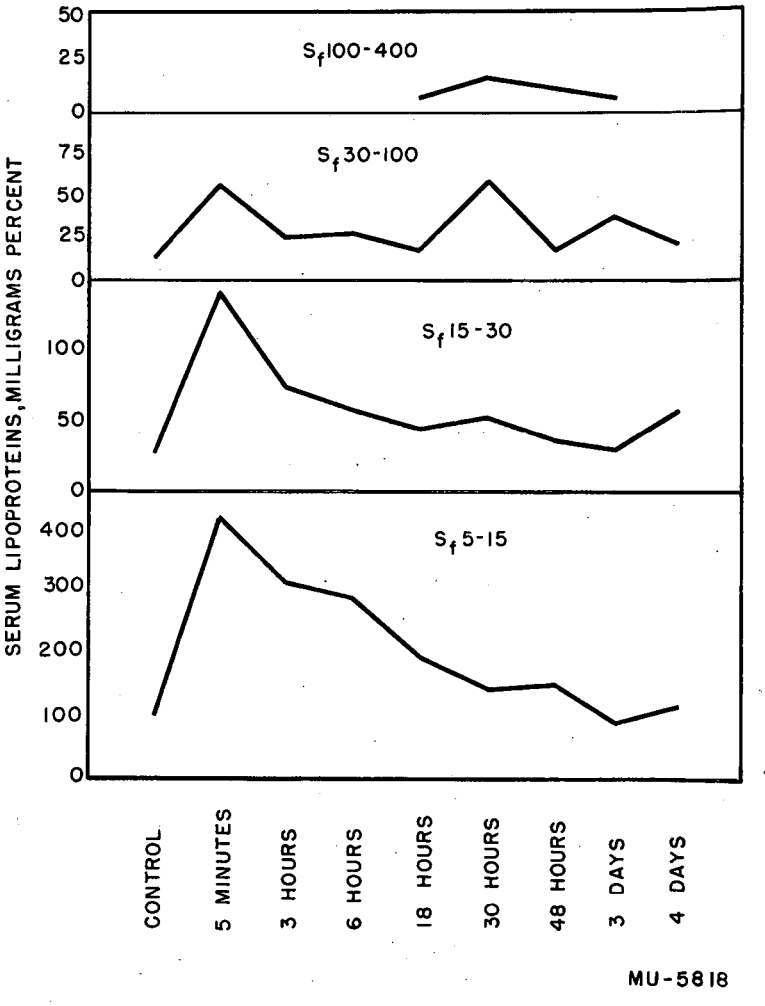
SERUM LIPOPROTEINS, MILLIGRAMS PERCENT
S_f100-400
S_f30-100
S_f15-30
S_f5-15
50
25
0
75
50
25
0
100
50
0
400
300
200
100
0
CONTROL
5 MINUTES
3 HOURS
6 HOURS
18 HOURS
30 HOURS
48 HOURS
3 DAYS
4 DAYS
MU-5818

Fig. 6 A

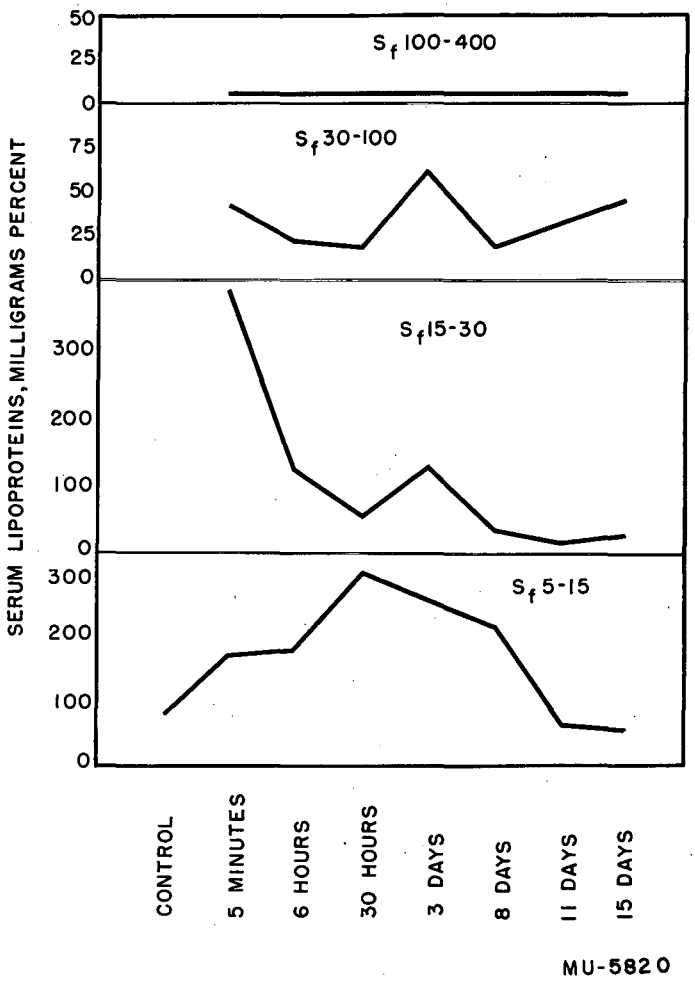
SERUM LIPOPROTEINS, MILLIGRAMS PERCENT
S_f 100-400
S_f 30-100
S_f 15-30
S_f 5-15
50
25
0
75
50
25
0
300
200
100
0
300
200
100
0
CONTROL
5 MINUTES
6 HOURS
30 HOURS
3 DAYS
8 DAYS
11 DAYS
15 DAYS
MU-5820

Fig. 6 B

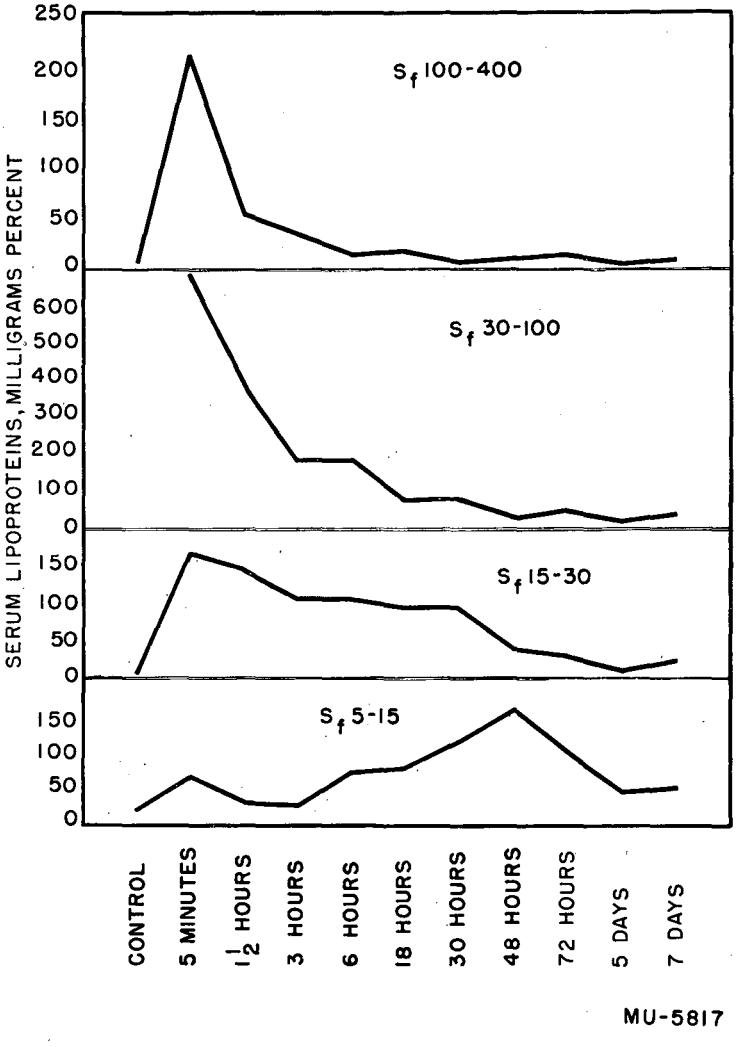
SERUM LIPOPROTEINS, MILLIGRAMS PERCENT
250
200
150
100
50
0
S_f 100-400
600
500
400
300
200
100
0
S_f 30-100
150
100
50
0
S_f 15-30
150
100
50
0
S_f 5-15
CONTROL
5 MINUTES
½ HOURS
3 HOURS
6 HOURS
18 HOURS
30 HOURS
48 HOURS
72 HOURS
5 DAYS
7 DAYS
MU-5817

Fig. 6 C

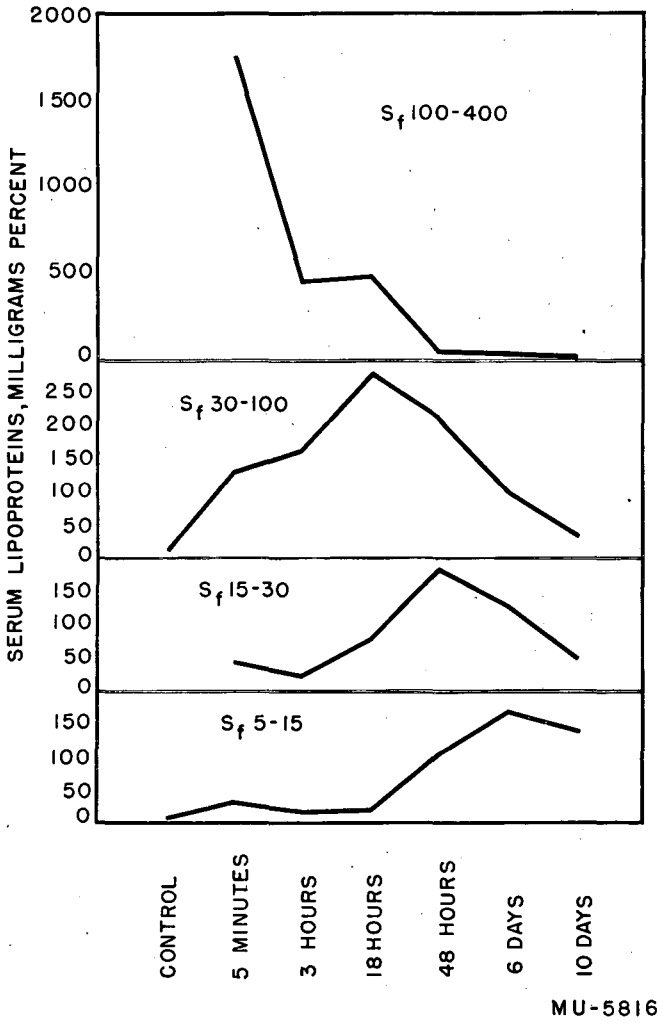
SERUM LIPOPROTEINS, MILLIGRAMS PERCENT
2000
1500
1000
500
0
S_f 100-400
250
200
150
100
50
0
S_f 30-100
150
100
50
0
S_f 15-30
150
100
50
0
S_f 5-15
CONTROL
5 MINUTES
3 HOURS
18 HOURS
48 HOURS
6 DAYS
10 DAYS
MU-5816

Fig. 6 D

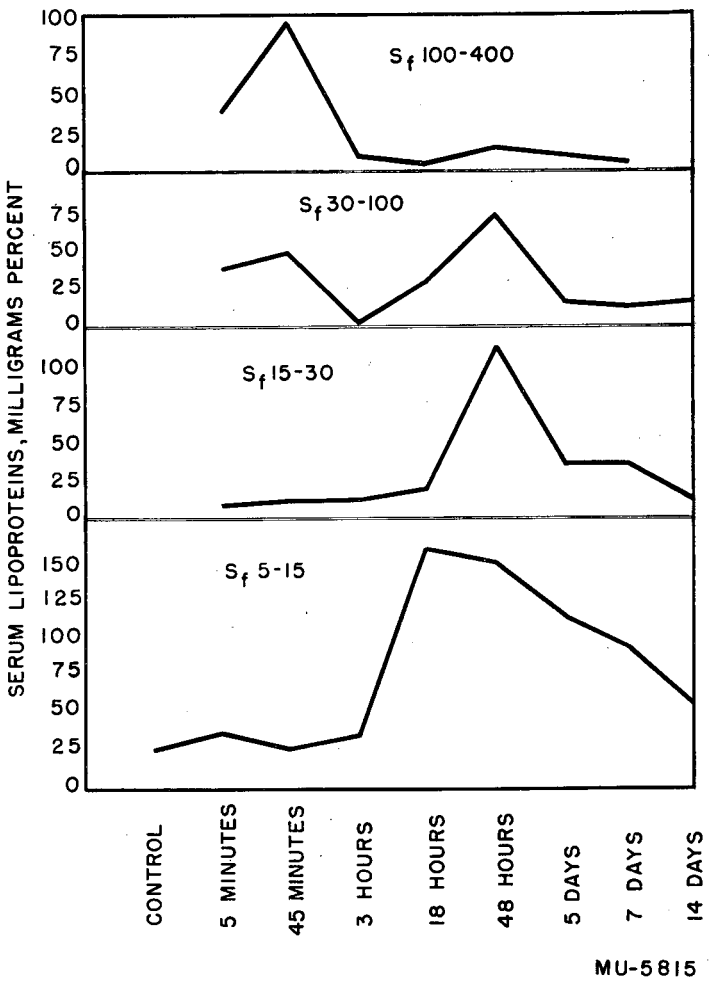
SERUM LIPOPROTEINS, MILLIGRAMS PERCENT
S_f 100-400
S_f 30-100
S_f 15-30
S_f 5-15
100
75
50
25
0
150
125
CONTROL
5 MINUTES
45 MINUTES
3 HOURS
18 HOURS
48 HOURS
5 DAYS
7 DAYS
14 DAYS
MU-5815

Fig. 6 E

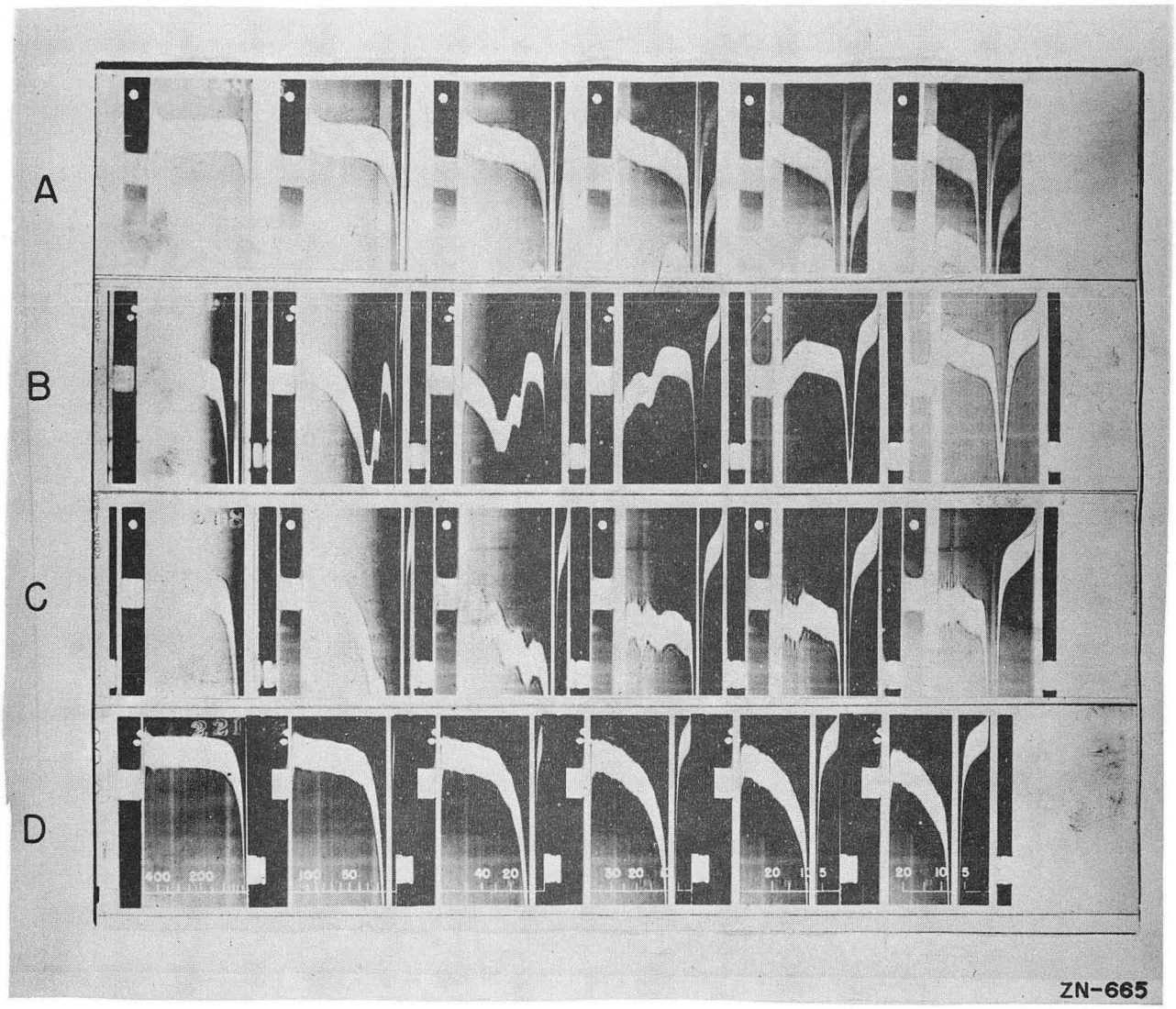

Fig. 7