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Elveda Smith (Name) for the Ph. D. (Degree) in Foods and Nutrition (Major)

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Although much information is available on lipids in serum and red cells, only limited data have been reported for white cells and platelets. In a recent investigation in this laboratory, the concentrations of cholesterol in the several blood fractions were compared. The high amounts of cholesterol found in white cell-platelet samples together with the wide variability in the proportions of free cholesterol stimulated an interest to study in more detail the lipid composition of the four blood fractions.

The concentrations of total lipids, the distributions of lipid classes, and the fatty acid compositions of cholesterol esters, triglycerides, and phospholipids were determined in serum, red cells, white cells, and platelets isolated from venous blood obtained from sixteen adult men and women. Supplementary analyses were made on nine samples of blood from one person at intervals over a four-week period.

Modifications of a published method made possible the

simultaneous separation of each of the formed elements; serum was obtained in the usual manner. Satisfactory procedures for total lipid extraction from each blood fraction were developed by selective combinations of individual steps of the classical ethanol-diethyl ether and chloroform-methanol methods. The proportional distribution of lipid classes in each blood fraction was quantitated by chemical analyses of cholesterol and lipid phosphorus and subsequent calculations. Lipid classes were isolated by preparative thin-layer chromatography. Methyl esters of the major classes were prepared by direct interesterification and analyzed by gas-liquid chromatography.

Concentrations of total lipid in sera and red cells were similar, averaging 603 and 555 mg per 100 ml, respectively. White cells and platelets contained two to three times as much, averaging 3051 and 2587 mg per 100 gm, respectively. The different blood fractions exhibited characteristic distributions of lipid classes. Serum was characterized by high cholesterol esters and triglycerides, together with phospholipids. All cellular components contained higher proportions of phospholipids and free cholesterol than serum. White cells and platelets contained higher amounts of cholesterol esters and free fatty acids than red cells. The fatty acid compositions of cholesterol esters, triglycerides, and phospholipids were not constant among the different blood fractions. The proportions of fatty acids in triglycerides were most similar. Greatest

variations in proportions of fatty acids occurred in cholesterol esters. Lipid classes in all cellular fractions were characterized by containing significant amounts of unidentified longer chain fatty acids which were essentially absent in serum.

Individual-to-individual variations among the sixteen subjects were greater than day-to-day variations of the one subject for total lipids in serum and red cells, all lipid classes in serum, and phospholipids in all blood fractions.

The concentrations of lipid in blood fractions showed no relationship to the age or sex of the subjects or to their assessed intakes of fat. Fat intakes were quite similar, however.

Differences in the distribution of lipid classes and fatty acid composition of lipid classes among the four blood fractions for these healthy subjects suggest the desirability of concurrent analyses of all blood fractions in studies of lipid metabolism in humans.

THE LIPIDS IN HUMAN BLOOD FRACTIONS

by

ELVEDA SMITH

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Professor of Foods and Nutrition

In Charge of Major

Head of Department of Foods and Nutrition

Dean of Graduate School

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INTRODUCTION

All types of lipids which are present in the tissues of the body occur in greater or lesser amounts in the blood. The blood is a mixture of serum, red cells, white cells, and platelets, and the lipids of the various fractions differ from each other in concentrations, properties, and functions. The serum is generally assumed to be most readily influenced by diet and nutritional processes. Total lipid concentration, the distribution of lipid classes, and the fatty acid composition of the classes all show some degree of fluctuation (30, 41). Although the total lipid and the distribution of lipid classes in red cells are more stable than in serum (4, 37), studies with animals have shown that the kind and amount of dietary fat affect the fatty acid composition of the different classes (29, 107). Less is known concerning the composition of lipids in white cells and platelets. However, it has been reported that certain disease conditions affect the amounts of total lipid and the distributions of lipid classes in all of the fractions of the blood (4, 13, 25, 30).

For many years, it was assumed that lipids normally present in blood were synthesized by the liver and other organs of the body and that blood merely acted as a transporting medium for lipids to and from other tissues. Contrary to this earlier belief, in 1959

studies using radioactive isotopes demonstrated that the white cells, or leucocytes, and platelets were active as sites of lipid synthesis. Synthesis of lipids by red cells, or erythrocytes, appeared to be limited to the young cells. Exchanges of lipid among blood fractions also were demonstrated by this technique (20, 48, 66).

In a recent investigation in this laboratory (43), the concentrations of cholesterol determined in serum, red cell, and white cell-platelet samples of thirty-three adult human subjects were compared. The amounts of cholesterol in serum and red cells were comparable to values reported in the literature, but the white cell-platelet samples contained a much higher concentration than values previously reported. Further, in contrast to the very limited individual differences in the proportion of free cholesterol in serum and red cells, the proportions in white cell-platelet samples showed wide individual variation. This investigation stimulated an interest to compare in more detail lipids in the several blood fractions.

In 1960, Nelken, Gilboa-Garber and Gurevitch (71) published a method for the simultaneous separation of white cells and platelets from small samples of blood. Several modifications of this method made possible the isolation of the formed elements of blood into three separate fractions: red cells, white cells, and platelets. Serum, of course, could be readily isolated. Satisfactory procedures for lipid extraction were then developed for each blood fraction by

adapting the classical ethanol-diethyl ether (55) and the chloroform-methanol methods (38). The proportional distribution of lipid classes in each fraction was quantitated by chemical analyses of free and total cholesterol and lipid phosphorus using suitable micromethods (86) and subsequent calculations. Preparative thin-layer chromatography was used to separate the total lipid mixtures into major lipid classes (60) and the types and percentages of fatty acids esterified in cholesterol esters, triglycerides, and phospholipids were determined by gas-liquid chromatographic analysis of the methyl esters after direct interesterification (89).

The lipids of serum, red cells, white cells, and platelets were analyzed for sixteen subjects, male and female, ranging in age from 23 to 66 years. The general diet patterns with particular attention to kinds and amounts of fat consumed were assessed. An additional study was made on nine samples of blood taken from one subject at intervals over a four-week period to investigate the degree of constancy or variability of lipids in the several blood fractions of the same individual.

REVIEW OF LITERATURE

Lipids in Blood Serum

Lipids in serum occur mainly as cholesterol esters, triglycerides or neutral fat, free fatty acids, free cholesterol, and phospholipids. Although under normal conditions the absolute amount of total lipid shows variation, individual constituents tend to maintain a characteristic and constant distribution (4, 30, 41). However, certain physiological factors, such as disease and differences in hormone secretion, do cause wide variations from the usual values (25, 30, 36, 101). The literature contains numerous reports on investigations of serum cholesterol which have been extensively reviewed (25, 26, 30, 52). Fewer data are available on the other lipid classes, but the importance of simultaneous investigations of all lipid components has been recognized, particularly in recent years (1, 27, 101).

Values for total lipid and the distribution of lipid classes, which have been reported by several investigators, are presented in Table 1. Most mean values for total lipid recorded over a thirty-year period ranged from 530 to 650 mg per 100 ml serum. Higher mean concentrations, 735 mg (72) and 836 mg (51), however, have been determined. Albritton (2) cites 397 to 722 mg per 100 ml serum as the range of standard values for humans. Slightly more

Table 1. Total Lipids and the Distribution of Lipid Classes in Serum Reported in the Literature

Investigator	Total Lipid	Lipid Classes: Cholesterol Esters	Free Cholesterol	Triglycerides plus Free Fatty Acids	Phospholipids
	mg/100 ml	Percent Total Lipid			
Man and Peters, 1933 (62)	659				34
Boyd, 1935 (12)	595	35	9	25	31
Page et al., 1935 (72)	735	34	11	31	25
Kornerup, 1950 (51)	836	32	7		21
West and Todd, 1951 (102)	530	34	9	27	31
Wilmot and Swank, 1952 (104)	591	37	10	18	36
Luddy et al., 1958 (57)		39	8	16	32
Hallgren et al., 1960 (41)	588	44	12	16	34
Lund et al., 1961 (58)	635	47	4		
Lund et al., 1961 (59)	568	62	5		
Svanborg and Svennerholm, 1961 (90)	629	34	10	14	35
Waxler and Craig, 1964 (101)	587			14	

than one-third of the total lipid usually are cholesterol esters, about one-third, phospholipids, one-fifth, triglycerides, and about one-tenth is free cholesterol. Although free fatty acid values are usually combined with triglycerides, the amount of free acids in serum has been reported as two to three percent of the total lipid (41, 57).

The fatty acid contents of cholesterol esters, triglycerides, and phospholipids of serum reported by several investigators are summarized in Table 2. Most of the values reported for the major fatty acids, palmitic (16:0), stearic (18:0), oleic (18:1), linoleic (18:2), and arachidonic (20:4), showed good agreement. Values which differed markedly, however, have been reported. Hanahan et al. (42) found serum triglycerides contained ten percent arachidonic acid whereas other investigators found two percent or less. The percentage of oleic acid in phospholipids reported by Luddy et al. (57), 36 percent, was twice as much as values generally found. The proportions of fatty acids reported appear to be characteristic to each class. In cholesterol esters, about half the fatty acids were linoleic, about one-fourth, oleic, and a little more than one-tenth, palmitic. In triglycerides, most of the fatty acids were palmitic and oleic, with slightly higher concentrations of oleic, and only about one-tenth, linoleic. In phospholipids, palmitic acid predominated, equalling about one-third of the total fatty acids, and about half of the total fatty acids were present as stearic, oleic, and linoleic

Table 2. Major Fatty Acids of Lipid Classes in Serum Reported by Several Investigators

Lipid Class	Percent Fatty Acids					Investigators
	16:0	18:0	18:1	18:2	20:4	
Cholesterol Esters	11-17	tr-4	21-30	40-52	4-8	Hallgren et al., 1960 (41); Hanahan et al., 1960 (42); Luddy et al., 1958 (57); Micheals et al., 1959 (67); Schrade, 1961 (83); Scott et al., 1964 (84); Swell et al., 1962 (91); Tuna and Mangold, 1963 (96); Tuna et al., 1958 (97)
Triglycerides	25-31	4-7	36-45	10-14	tr-2	Hallgren et al., 1960 (41) Luddy et al., 1964 (57); Schrade, 1961 (83); Swell et al., 1962; Tuna and Mangold, 1963 (96)
Phospholipids	28-37	12-14	14-19	14-21	4-9	Dole et al., 1959 (31); Hallgren et al., 1960 (41); Schrade, 1961 (83); Scott et al., 1964 (84); Swell et al., 1962 (91)

acids in relatively comparable amounts.

Lipids in Red Cells

The lipids of red cells differ from those in serum in concentration, properties, and functions. They are part of the fixed framework of the cell and their function is primarily of structural importance. Until recently, it was generally assumed that the red cell lipids, in contrast to serum lipids, were quite stable to the influences of nutrition (4, 37). However, in several animal studies it was shown that the nature of dietary fat, the proportion of fat, and the tocopherol intake all affected the fatty acid composition of lipids of red cells, particularly the fatty acids in phospholipids (23, 47, 68, 73, 99).

The earliest values for lipids of red cells determined by reasonably modern methods were reported by Abderhalden in 1908 (30). From analyses of several of the lipid components in the cells of a number of different animal species, he found that the concentrations of cellular lipid components were quite different from concentrations of the same components in plasma or serum. In 1916, Bloor (7) studied the distribution of lipids in human blood. He reported that the "lecithin" content of red cells was approximately double that of plasma, whereas cholesterol and "total fatty acid" values were almost always lower than those in the plasma. The

neutral fat component was practically absent from the erythrocyte. Following these early investigations, the lipids of red cells have been studied under a variety of conditions and a number of extensive reviews have been published (4, 8, 19, 30).

The mean values of total lipid in red cells and the distribution of lipid classes, which have been reported by several investigators, are given in Table 3. The values for total lipid ranged

Table 3. Total Lipids and the Distribution of Lipid Classes in Red Cells Reported in the Literature.

		Lipid Classes			
Investigator	Total Lipid mg/100 ml	Choles. Esters	Free Choles.	Triglycerides plus Free Fatty Acids	Phospho-lipids
		Percent Total Lipid			
Erickson et al., 1937 (33)		7	23	12	58
Wilmot and Swank, 1952 (104)	590	1	22	11	67
Prankerd, 1955 (77)		4	20	11	65
Reed et al., 1958 (81)			22		69
Barkhan et al., 1960 (3)					67
Reed et al., 1960 (82)	604		23		65
Troup et al., 1961 (92)	584		23		69
Farquhar and Ahrens, 1963 (35)	510				58

from 500 to 600 mg per 100 ml red cells. Albritton (2) cites 411 to 781 mg per 100 ml as the normal range in humans. The lipid class present in highest concentration is phospholipids, which comprises about two-thirds of the total lipid. One-fourth of the lipid is cholesterol, which is mainly unesterified, and about one-tenth is triglyceride.

Until recently, only a few isolated attempts had been made to identify the fatty acids associated with the lipids of human red cells. The fatty acid composition of the total lipid sample was reported by several investigators (46, 50, 70). Analyses of the individual lipid classes, however, are limited (35, 42, 100). Data reported by several investigators on the major fatty acids in cholesterol esters, triglycerides, and phospholipids are presented in Table 4. The proportions of the major fatty acids found in each lipid class are generally similar to those found in serum, although the exact percentages differ. Of particular note is the high amount of arachidonic acid present in phospholipids.

Table 4. Major Fatty Acids of Lipid Classes in Red Cells Reported by Several Investigators

	Percent Fatty Acids						
Lipid Class	14:0	16:0	18:0	18:1	18:2	20:4	Investigator
Cholesterol Esters	9	11	4	13	63	tr	Hanahan et al., 1960 (42)
Triglycerides		31	13	30	12	2	Hanahan et al., 1960 (42)
Phospholipids		32	15	20	11	20	Farquhar and Ahrens, 1963 (35)
Phospholipids	1	25	19	16	11	15	Ways and Hanahan, 1964 (100)

Lipids in White Cells

Only limited information is available concerning the concentrations of total lipids and the distribution of lipid components in the white cells. The data obtained in a few studies on human leucocytes are supplemented by studies on leucocytes of animals. Boyd reported two different ranges for the total lipid content in the white cells of normal subjects: in 1933 (9), 1029 to 3413 mg per 100 gm for eight young women; and, in 1936 (15), 688 to 2142 mg per 100 gm for thirty subjects. Boyd (15) also found essentially no difference between the lipid content in normal and leukemic cells. Amounts of total lipids comparable to those found in human cells have been determined for rabbit leucocytes. Boyd and Stevenson (17) found values that ranged from 894 to 2433 mg per 100 gm of cells and Burt and Rossiter (22) stated that rabbit polymorphonuclear leucocytes averaged 1764 mg of lipid per 100 gm. On the basis of the dry weight of cells, the total lipid contents have been reported as 8.7 ± 2.9 percent for polymorphonuclear leucocytes of rabbits (32) and 22 percent for leucocytes of horses (34).

The distributions of lipid classes in white cells, as found in the literature, are presented in Table 5. The concentrations of lipid components are quite similar to those in red cells with about two-thirds as phospholipids, but only about one-fifth is present as

Table 5. Total Lipids and the Distribution of Lipid Classes in White Cells of Humans and Rabbits Reported in the Literature

Source	Total Lipid	Lipid Classes: Choles. Esters	Free Choles.	Trigly-cerides	Phospho-lipids	Investigator
	mg/100 gm	Percent Total Lipid				
Human - Normal						
Leucocytes	1710	11	11	31	47	Boyd, 1933 (9)
Leucocytes	1447	6	16	14	60	Boyd, 1936 (15)
Leucocytes		3	12	4	77	Malamos et al., 1962 (61)
Rabbit						
Leucocytes	1860	8	16	30	46	Boyd and Stevenson, 1937 (17)
Polymorphonuclear Leucocytes	1764	1	13	30	55	Burt and Rossiter, 1950 (22)
Polymorphonuclear Leucocytes		9	5	22	62	Elsbach, 1959 (32)

cholesterol, more of which is generally esterified, and there are more triglycerides. In a few other studies, concentrations of cholesterol and phospholipids have been determined but not with total lipid values which would allow a calculation on a percent basis. Mean values for total cholesterol of 300 mg (9) and 700 mg per 100 gm cells (43) and a range for phospholipids of 400 to 1400 mg per 100 gm (14) would support the higher concentrations summarized in the table. In addition, higher phospholipid and free cholesterol values also have been associated with increased activity of cells (10, 11, 13, 16).

To the knowledge of the author, no data have been reported on the fatty acid composition of lipids in human leucocytes, either of total or of any class. Data were reported by Elsbach (32) for polymorphonuclear leucocytes in rabbits, and the results of his analyses are presented in Table 6.

Table 6. Major Fatty Acids of Total Lipids and of Lipid Classes in Rabbit Leucocytes (32)

	Percent Fatty Acids										
Sample	12:0 14:0 15:0	16:0	16:1	17:0	18:0	18:1	18:2 18:3	?	20:0 20:1 20:2 20:3	20:4 20:5	22:0
Whole Lipid Extracts	3	23	4	3	14	17	32	3	tr	3	tr
Triglycerides plus Cholesterol Esters	3	28	5	3	12	29	21		2	tr	
Phospholipids	1	20	2	2	13	15	38	2	2	3	1

Lipids in Blood Platelets

In the only studies on lipids in platelets of humans or animals, the total lipid content has been reported as the percentage of the dry material of the cells, Table 7. Horse platelets have been found to contain 12 (34) and 17 percent (24), whereas values for bovine platelets were 12 and 23 percent, with two different methods of extraction (108). Several investigators have reported values for human platelets to range from 15 to 17 percent (3, 34, 53), which were similar to those determined by Chargaff (24) in horse platelets, 17 percent. In a more recent study, two different values, 15 and 25 percent, were obtained by two different extraction procedures (108).

The distributions of lipid classes, summarized in Table 7, appeared to be similar to those found in both red cells and white cells, but data are less detailed. However, in platelets, the phospholipids are present in even higher amounts equalling about three-fourths of the total lipid concentration.

There is much current interest in the relationship of the phospholipid class of platelet lipids to the clotting mechanism. The fatty acid composition of the individual phospholipid components have been reported (6, 64, 65), although no data are available concerning the fatty acid composition of the total phospholipid class or of any other class.

Table 7. Total Lipids and the Distribution of Lipid Classes in Platelets of Humans and Animals Reported in the Literature

Source	Total Lipid % Dry Wt.	Lipid Classes: Choles. Esters (Percent Total Lipid)	Free Choles. (Percent Total Lipid)	Triglycerides (Percent Total Lipid)	Phospholipids (Percent Total Lipid)	Investigator
Human	16	12	13	0	75	Lee and Erickson, 1938 (53)
	15	4	16	5	74	Erickson et al., 1939 (34)
	15[1]	1	19		72	Woodside and Kocholaty, 1960 (108)
	25[2]	1	19		72	Woodside and Kocholaty, 1960 (108)
	17		24		76	Barkhan, 1961 (3)
Horse	12		14			Erickson et al., 1939 (34)
	17					Chargaff et al., 1936 (24)
Bovine	12[1]	1	19		69	Woodside and Kocholaty, 1960 (108)
	23[2]	1	19		69	Woodside and Kocholaty, 1960 (108)

[1]Percentage of total lipids extracted by method of Hsiao.
[2]Percentage of total lipids extracted by method of Folch et al.

ANALYTICAL METHODS

Isolation of Blood Fractions

Methodology Studies

Five different methods for the separation of white cells and platelets from blood were investigated. The methods included: differential centrifugation following the procedure of Bessey, Lowry and Brock (5); differential sedimentation according to the procedure of Li and Osgood (54); differential sedimentation following the procedure of Gold and Cole (39); hemolysis of red cells as outlined by Szilard (85); and a combination of differential centrifugation and sedimentation following the procedure of Nelken, Gilboa-Garber and Gurevitch (71). Criteria for evaluating the various methods were the visual observation of the size of the sample of white cells or platelets recovered, the number of red cells contaminating the sample, and microscopic observations of the morphology of the cells. No attempt was made to determine the percent recovery of the white cells or platelets or to determine quantitatively the ratio of these cells to red cells present.

Although the method of Bessey, Lowry and Brock (5) for the separation of the white cell-platelet sample had been used extensively in this laboratory, difficulty had been experienced in obtaining the sample free from contaminating red cells. Repeated centrifugation

of the plasma-anticoagulant suspension of cells resulted in purer samples but the loss of cells was high. Varying the concentration of the anticoagulant, which was added to the blood, did not improve the separation or the recovery of the cells. In addition, white cells and platelets could not be obtained as two separate samples. Good recovery of the white cell-platelet sample from blood was achieved when phytohemagglutinin was used as the sedimenting agent for red cells according to the method of Li and Osgood (54), but again the white cells and platelets were not isolated as separate fractions. Gold and Cole (39) published a method of leucocyte separation using polyvinylpyrrolidone. The method proved to be unsatisfactory since the sample of white cells could not be obtained free from a brown precipitate. Several attempts to isolate white cells by selective hemolysis of red cells with acetic acid-tartaric acid (85) were not successful. When potassium hydroxide was added to neutralize the hemolyzed blood mixture, a brown precipitate formed which rendered the sample useless.

In 1960, Nelken, Gilboa-Garber and Gurevitch (71) published a method which combined differential centrifugation and sedimentation in a series of steps for the simultaneous separation of platelets and of white cells from the same sample of blood. A modification of this method was satisfactory for the isolation of white cells, platelets, and red cells. The lighter platelets, which were separated

from the other formed elements by differential centrifugation, were obtained almost free from other cells. However, the white cells, recovered after the red cells were sedimented with dextran, contained a high proportion of red cells as well as some platelets. Nelken et al. used distilled water to hemolyze the red cells as a technique of purifying white cell samples, but this procedure did not result in adequate removal of red cells and was not used. To effect good separation of the white cells, the cell suspension was concentrated in a small volume of physiological saline and then drawn into fine capillary tubing which was sealed and centrifuged. In using the capillary tubing, however, the concentration of cells in the suspension could not be too high or the red cells became trapped by white cells and were not thrown to the bottom of the capillary tube. As a result, no sharp boundary of red and white cells occurred.

Another problem which was encountered in the isolation of white cells was in vitro clumping. This problem had been recognized by other investigators (40, 93, 94, 98). Tullis (93) stated that clumping of this type was seen most commonly when the cells were exposed to red cell sedimenting agents. In this study, when the white cell sample was centrifuged in the fine capillary tubing, large aggregates of cells did form which could not be dispersed by vigorous mixing of the cell suspension. However, it was found that the aggregates of white cells could be broken more readily if the

sample was laked in water and frozen. Several investigators have also recommended that the glassware be coated with silicone to minimize the in vitro clumping of cells (40, 76, 93).

The red cell sample which was obtained by sedimentation in this procedure was compared with red cells which were isolated by the method of Burch, Bessey and Lowry (21) which had been used previously in this laboratory. The total lipid and cholesterol contents of both samples were essentially identical.

Method

Equipment

1. Syringe pipet, 30 ml.
2. Hypodermic needles, B-D Champion Luer-Lok veterinary needles, hyperchrome stainless steel, 20 gauge.
3. Tubes: 20 x 150 mm, 10 x 75 mm, 12 x 100 mm, 7 x 50 mm.
4. Test tube racks.
5. Refrigerated centrifuge with automatic timing device.
6. Capillary tubing, glass, 1 x 80 mm.
7. Volumetric flasks, 1 ml.
8. Buzzer, a mechanical agitator.
9. Diamond point pen.
10. Micro hematocrit set, Critoseal.[1]

[1] Aloe Scientific, 5655 Kingsbury, St. Louis, Missouri.

11. Pipet, transfer, Wintrobe.

12. Christian Becker chainomatic balance, No. 14.

Reagents

1. Anticoagulant, 1 percent solution each of disodium ethylene diamine tetra-acetic acid (Na_2-sequestrene) and Triton W.R. 1339[1] (oxyethylated tertiary octyl phenol formaldehyde polymer) in 0.7 percent sodium chloride solution. One gram each of Na_2-sequestrene and Triton W.R. 1339 were dissolved in 0.7 percent sodium chloride and the solution was made up to 100 ml.

2. Sodium chloride solution, 0.7 percent. Seven-tenths gram sodium chloride was dissolved in redistilled water and made up to 100 ml.

3. Physiological saline, 0.9 percent sodium chloride solution. Nine-tenths gram sodium chloride was dissolved in redistilled water and made up to 100 ml.

4. Dextran solution (Dextran IV, 150-200,000 M.W.), 6 percent in physiological saline. Six grams dextran was dissolved in physiological saline and made up to 100 ml. The dextran solution was stored in the cold.

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[1] Winthrop Laboratories, Special Chemical Department, 1450 Broadway, New York 18, New York

Procedure

Serum

About 5 ml of whole blood was placed in a 12 x 100 mm tube, allowed to clot, and then centrifuged at 1300 g for one hour (0°C). A measured volume of serum was transferred to a 10 x 75 mm tube. The tube was stoppered and the sample was stored at -5°C until analysis.

Blood Cells

The red cell, white cell, and platelet samples were isolated from 20-25 ml of whole blood. The blood was mixed with anticoagulant in the proportion of nine parts blood to one part anticoagulant. The blood-anticoagulant mixture was mixed thoroughly in a 20 x 150 mm tube by inverting the tube several times with a gentle motion to prevent foaming.

Blood platelets. The mixture was centrifuged (0°C) at 110 g for seven minutes to concentrate the heavier red cells and white cells in the bottom of the tube. The platelet-rich plasma supernatant was transferred to 7 x 50 mm tubes and the suspension was centrifuged at 800 g for ten minutes to pack the platelets. The platelet-poor plasma supernatant above the packed platelets was drawn off and returned to the original tube containing the red and white cells.

The platelets were dispersed in a small volume of physiological

saline and the sample was quantitatively transferred to a previously weighed one-milliliter volumetric flask. The sample was centrifuged at 1300 g for ten minutes, and the physiological saline above the packed platelets was drawn off as close to the cells as possible. The flask was turned upside down to drain and the wet weight of the platelets was obtained by weighing to the nearest 0.1 mg. The platelets were laked in 1 ml redistilled water, the flask was stoppered, and the sample was held at -5°C until analysis.

White cells. After the platelet-poor plasma supernatant was returned to the tube containing the red cells and the white cells, the cells and plasma were mixed well. To this mixture was added one-fourth volume of a six percent dextran solution which had previously been warmed to 37°C. The dextran was mixed with the remaining cell sample by pouring the dextran-red cell-white cell mixture from one tube to another several times. The mixture was allowed to stand at room temperature for 25 minutes at a 45° angle to accelerate the sedimentation of red cells and then the tube was placed in an upright position at a 90° angle for five minutes.

The supernatant plasma containing the white cells plus some red cells and platelets was transferred to a 12 x 100 mm tube. The white cell-enriched mixture was centrifuged at 1300 g for ten minutes to pack the cells. The supernatant solution was drawn off and discarded, and the packed cells were dispersed in approximately

0.5 ml of physiological saline. The suspension was drawn into fine capillary tubing, one end of the tube was plugged with Critoseal, and the samples were centrifuged at 1300 g for ten minutes. The capillary tubing was broken at the white cell-red cell boundary. The packed white cells were quantitatively transferred to a previously weighed one-milliliter flask by blowing and repeated washings. The cell mixture was centrifuged, weighed, and stored according to the procedure described for platelets.

Red cells. The original tube containing the red cells was centrifuged at 1300 g for one hour. The supernatant solution above the packed cells and any remaining white cells were removed. The red cells were measured volumetrically and laked in two volumes of redistilled water. The tube containing the laked cells was stoppered and the sample was held at -5°C until analysis.

Extraction of Total Lipids from Blood Fractions

Methodology Studies

Two methods for the extraction of total lipids were investigated. The method described in detail by Lis and Okey (55), which uses an initial hot ethanol extraction, a second extraction with ethanol-diethyl ether, and a final refluxing with diethyl ether in a Soxhlet apparatus, was compared with the method of Folch et al. (38), which uses two successive hot extractions with a chloroform-methanol

solvent system. Lipids were extracted from duplicate serum samples and the methods were compared as to: 1) the quantitative extraction of total lipid and the degree of degradation; 2) the relative rates of extraction of the different lipid components with successive extraction steps; and 3) the suitability of each procedure to the extraction of lipids from cells as well as serum.

The weights of the total lipid extracted by both methods were comparable which agreed with data reported by others (49, 88). However, thin-layer chromatographic analysis of the total lipid mixtures showed that some degradation occurred in the sample extracted by the ethanol-diethyl ether extraction, but little in the samples extracted by the Folch et al. method. To determine when the degradation occurred, the individual lipid extracts obtained in the Lis and Okey method were studied by thin-layer chromatography. It was found that during the final step breakdown of the lipid components occurred but the degradation could be held at a minimum if the solvent in the Soxhlet apparatus was replaced with fresh solvent early in the reflux period.

Thin-layer chromatographic analysis of the individual extracts of serum lipids obtained by both methods showed that different solvent combinations extracted certain lipid classes more readily than others. Using the ethanol-diethyl ether extraction, it was found that the initial ethanol extract contained all of the free cholesterol and

most of the phospholipids and cholesterol esters as well as some triglycerides and free fatty acids. The ethanol-diethyl ether extraction contained some triglycerides, free fatty acids, and phospholipids and a small amount of cholesterol esters. The extract that was obtained, after three and one-half hours of refluxing with diethyl ether contained triglycerides, free fatty acids, some cholesterol esters and phospholipids, and products that had been altered during the extraction. The distribution of classes in the two solvent extracts obtained by the method of Folch et al. showed that all of the cholesterol esters and triglycerides and most of the phospholipids and free cholesterol were extracted initially from the sample. The second extraction of the precipitate contained small amounts of phospholipids and free cholesterol, whereas comparable amounts of free fatty acids were present in both the initial and the second extraction of the sample.

In extracting lipids from red cells, white cells, and platelets, free cholesterol and phospholipids were generally extracted with as much ease as from serum. However, cholesterol esters and triglycerides were less readily extracted and at more diverse rates with the different solvent systems. These lipid components in white cells and platelets were particularly resistant to extraction and continued to be extracted through each step of the procedures.

When the individual lipid extracts of all of the formed elements

obtained by the method described by Lis and Okey were analyzed by thin-layer chromatography, all of the free cholesterol and most of the phospholipids were present in the initial ethanol extract. The distribution of the other lipid classes among the three extracts differed for the three types of cells. Although most of the cholesterol esters and triglycerides were extracted from red cells with ethanol, small amounts of these lipid classes as well as traces of phospholipids were present in the two succeeding extracts. Comparable amounts of free fatty acids were present in all three extracts. In contrast, cholesterol esters, triglycerides, and free fatty acids in white cells and platelets were extracted in approximately the same amounts with the three solvents. Using the Folch et al. solvent system for the extraction of lipids from red cells, most of the cholesterol esters and triglycerides were extracted initially, whereas free cholesterol, free fatty acids, and phospholipids were present in both extracts in quite high concentrations. On the other hand, the analyses of the extracts from white cells and platelets showed that nearly all of the free cholesterol and phospholipids were extracted initially, but comparable amounts of cholesterol esters, triglycerides, and free fatty acids were found in both extracts.

From the analyses of the individual lipid extracts obtained by both methods, particularly those from white cells and platelets, the effectiveness with which total lipid was extracted from cell samples

by either method was questioned. It was concluded that a selective combination of the two methods with modifications for the different blood fractions would be most satisfactory.

The procedures adopted involved initial extractions with chloroform-methanol of varying number and temperature followed by diethyl ether extractions of the precipitates of the formed elements for different lengths of time. These modifications were based on several factors. First, phospholipids were more soluble in chloroform-methanol than in ethanol-diethyl ether and nearly total extraction of the phospholipids occurred in the initial extraction of all samples except red cells. Second, cold chloroform-methanol, which was used in the first extraction of all samples, reduced the amount of pigment extracted from red cells and decreased as much as possible the exposure to heat of lipids of all fractions. Third, refluxing the precipitates from the formed elements with diethyl ether in the Soxhlet apparatus for varying lengths of time insured almost complete extraction of lipids. Finally, degradation of lipid components was held to a minimum when the diethyl ether extract was replaced with fresh solvent within two hours after the refluxing was started.

The effectiveness of the extraction procedures adopted were verified by thin-layer chromatography. Plates 1-4 show the distribution of lipid classes and the relative amounts of each lipid class which were extracted in successive steps of the procedure for each

blood fraction. For these chromatograms, the extractions were carried out at least one step beyond the procedures finally adopted.

In Plate 1, it is apparent that lipids of serum were extracted almost totally when the sample was treated initially with cold chloroform-methanol. Small amounts of lipid were extracted from the precipitate with hot solvent and only negligible amounts were extracted by refluxing with diethyl ether. Although nearly total extraction of lipid from red cells occurred when the sample was extracted three times with cold chloroform-methanol, Plate 2, there was an additional amount of lipid, primarily free fatty acids, which was extracted by diethyl ether after six hours. A similar small amount was extracted with an additional 12 hours of refluxing. An unidentified component (U) which appeared at the solvent front of the chromatogram also was extracted from red cells with diethyl ether. White cell and platelet samples were extracted initially with cold solvent and then the precipitate was extracted with hot solvent three times, Plates 3 and 4. Large amounts of lipid were present in each extract. Nearly total extraction occurred after 15 to 20 hours of refluxing the precipitate with diethyl ether. A negligible amount of lipid was extracted with an additional eight hours of refluxing. As with red cells, an unidentified component was extracted from white cells and platelets with diethyl ether. Although the unidentified component in these illustrations appears to be present in high

Plates 1 - 4.

1. Extraction of total lipids from serum: I, sample extracted with cold chloroform-methanol; II, precipitate extracted with hot chloroform-methanol; III-IV, successive refluxing of the precipitate with diethyl ether for 2 and 4 hours, respectively.

2. Extraction of total lipids from red cells: I, sample extracted with cold chloroform-methanol; II-III, precipitate extracted with cold chloroform-methanol; IV-VI, successive refluxing of the precipitate with diethyl ether for 2, 4, and 12 hours, respectively.

3. Extraction of total lipids from white cells: I, sample extracted with cold chloroform-methanol; II-IV, precipitate extracted with hot chloroform-methanol; V-VIII, successive refluxing of the precipitate with diethyl ether for 2, 4, 12, and 8 hours, respectively.

4. Extraction of total lipids from platelets: I, sample extracted with cold chloroform-methanol; II-IV, precipitate extracted with hot chloroform-methanol; V-VIII, successive refluxing of the precipitate with diethyl ether for 2, 4, 12, and 8 hours, respectively.

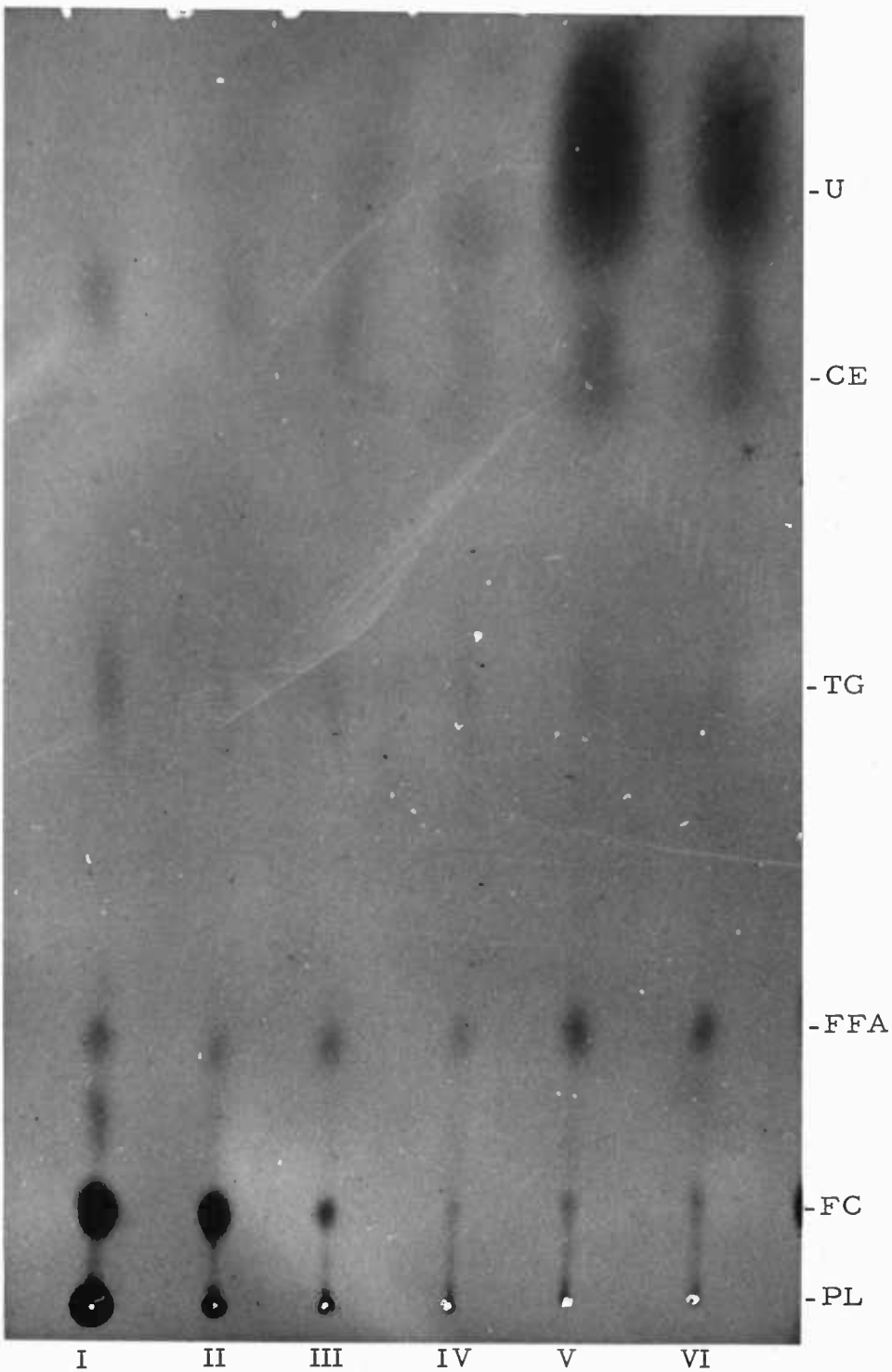

Plate 2.

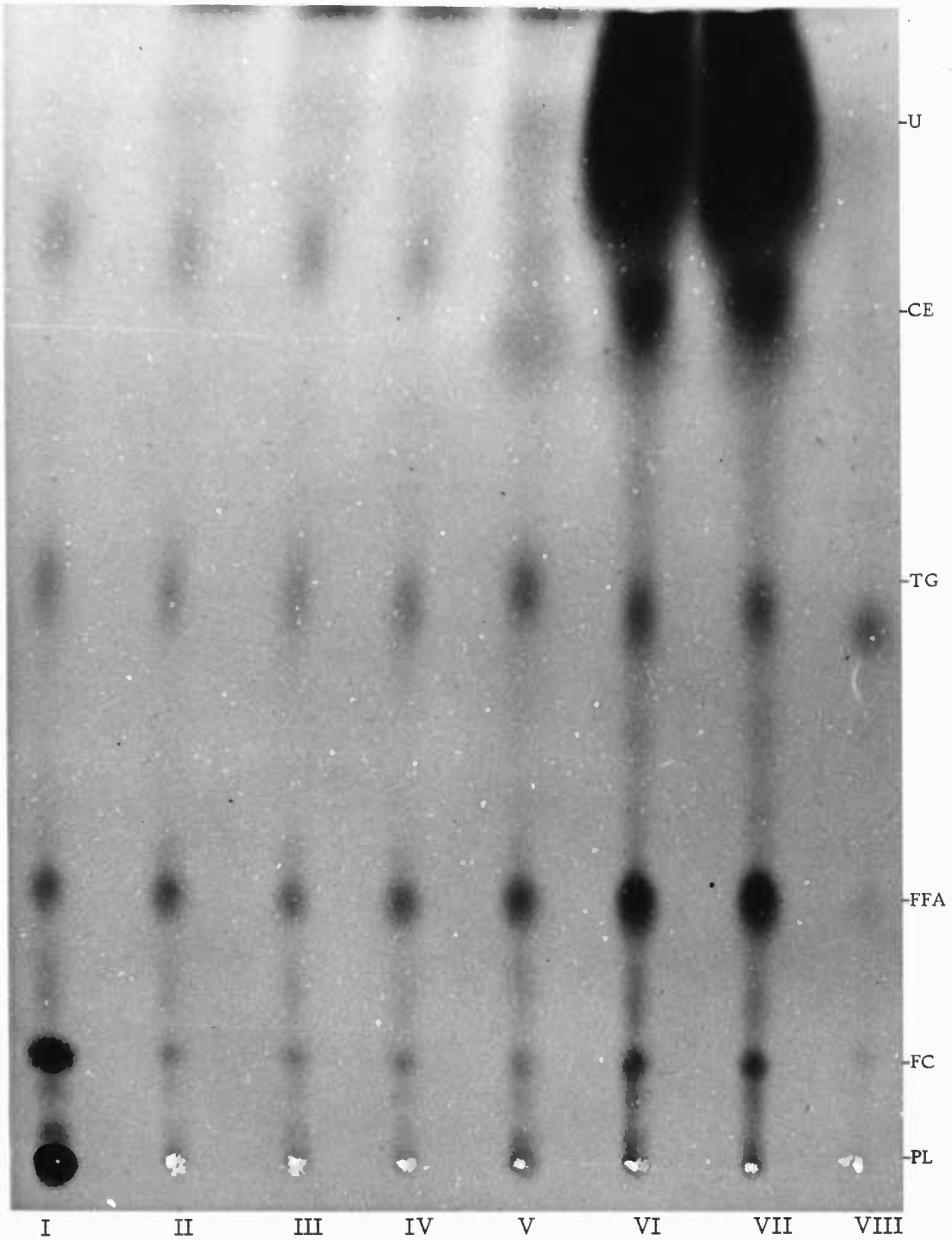

Plate 3.

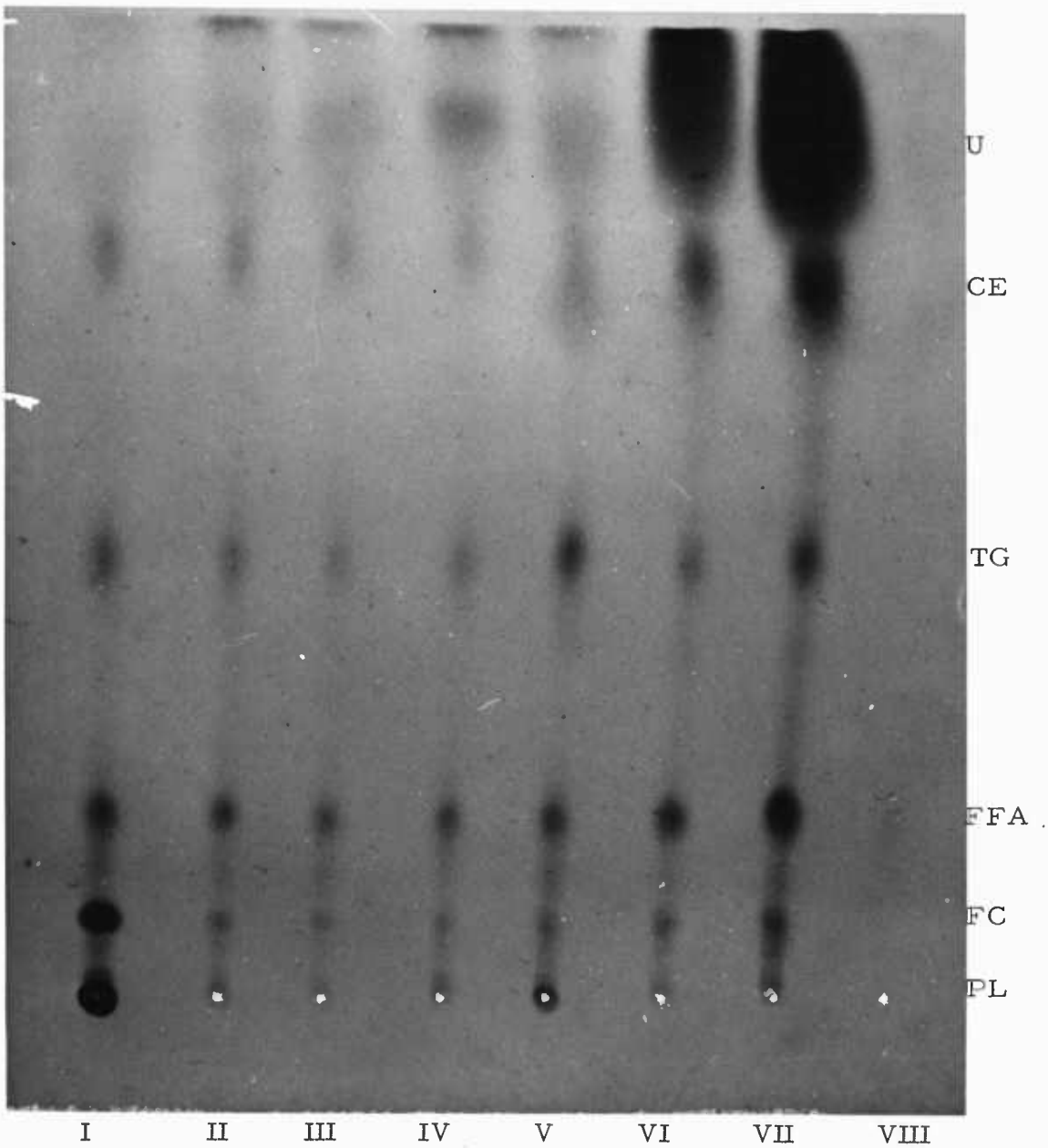

Plate 4.

concentration, the component actually is only a small proportion of the total extract since it is diluted when the individual extracts are combined. In the preparation of these plates, equal amounts of the various extracts were chromatographed to better illustrate the effectiveness of the extraction procedures.

Method

Equipment

1. Magnetic stirrer.
2. Fat extraction apparatus: heating unit and micro Soxhlet apparatus.
3. Glass manifold with stopcocks for controlling nitrogen gas flow.
4. Beakers, pyrex: 100, 150, 400 ml.
5. Erlenmeyer flasks: 125, 250 ml.
6. Filter paper, Whatman No. 1, 43. The paper was treated for fat extraction.
7. Extraction thimbles, Whatman, single and double thickness, 10 x 50 mm. The thimbles were treated for fat extraction.
8. Rinco rotation evaporator.[1]
9. Teflon Sleeves, Ⓣ joint 24/40.[2]

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[1] Rinco Instrument Company, Inc., 503 South Prairie Street, Greenville, Illinois.

[2] LaPine Laboratory Supplies, 6001 South Knox Avenue, Chicago 29, Illinois.

10. Flasks, round bottom, T joint 24/40:100, 250 ml.
11. Conical centrifuge tubes, graduated to 15 ml.
12. Volumetric flasks, 1 ml.
13. Vials, glass with screw cap, 15 x 45 mm, 1 dram.
14. Micro porous boiling chips.
15. Mettler micro balance, Model M5.
16. Evaporating dish and glass pestle.

Reagents

1. Chloroform, distilled. Five-tenths to one percent ethanol was added as a preservative. Chloroform was stored in the cold.
2. Methanol, absolute. Methanol was distilled over potassium hydroxide.
3. Skelly Solve B (petroleum ether), distilled and the fraction distilling between 66-69°C was collected.
4. Diethyl ether, Mallinckrodt's purified for fat extraction. Diethyl ether was stored in the cold.
5. Sodium sulfate, anhydrous.
6. Nitrogen gas.

Procedure

Serum Total Lipids

The frozen serum sample was warmed to 37°C in a water bath for 30 minutes, cooled to room temperature, and mixed well. One

volume was added dropwise to 20 volumes of chloroform-methanol (2:1, v/v) in a beaker while the solvent was kept in motion by use of a magnetic stirrer. The lipid extract was filtered through fat-free filter paper into an Erlenmeyer flask. The precipitate was washed into the beaker with 20 volumes of fresh solvent and the mixture was heated to boiling, cooled, and filtered into the original filtrate. Twenty percent by volume of redistilled water was added to the chloroform-methanol lipid extract. The water and solvent were mixed thoroughly, the flask was stoppered with a cork stopper, and the mixture was allowed to stand until two distinct layers separated and became clear. The water layer, which was on top, was removed by suction and discarded. Anhydrous sodium sulfate was added to the lipid extract to dry the solvent. The flask was flushed with nitrogen and stoppered with a cork stopper, and the sample was held overnight at -5°C.

The next day, the chloroform-methanol extract was filtered through fat-free filter paper into a round bottomed flask to remove the sodium sulfate. The sample was evaporated just to dryness under vacuum using a Rinco rotating evaporator while the flask was held in a warm water bath (50-60°C). The lipid was immediately dissolved in a small volume of petroleum ether and was quantitatively transferred to a 15-milliliter conical centrifuge tube. The petroleum ether was evaporated to less than 1 ml under a stream of

nitrogen while the tube was held in a warm water bath, and then the sample was transferred quantitatively to a one-milliliter volumetric flask. An exact aliquot of total lipid extract was weighed to 0.001 mg and the amount of total lipid was calculated.

Red Cell Total Lipids

The frozen sample of laked red cells was warmed to 37°C in a water bath for 30 minutes, cooled to room temperature, and mixed well. One volume of laked cells (equivalent to one part packed red cells and two parts water) was added dropwise to 20 volumes of chloroform-methanol (2:1, v/v) in a beaker while the solvent was kept in motion by use of a magnetic stirrer. The lipid extract was filtered through fat-free filter paper into an Erlenmeyer flask. The precipitate, which formed a putty-like mass, was air dried, ground to a powder, and re-extracted twice with 10 volumes of fresh solvent as suggested by Phillips and Roome (75) and Dawson et al. (28). The filtrates were added to the original extract and 20 percent by volume of redistilled water was added. The water and extract were mixed thoroughly, the flask was stoppered, and the mixture was allowed to stand until two layers separated and became clear. The water layer was removed and discarded, the solvent was dried, and the sample was stored according to the procedures outlined for serum.

The red cell precipitate was transferred to an extraction thimble and extracted with 20 ml of diethyl ether in the Soxhlet

apparatus in a nitrogen atmosphere for six hours. After two hours, the solvent plus the lipid was removed, fresh solvent was added to the apparatus, and the refluxing was continued for four hours. The two diethyl ether extracts of lipid from the red cell precipitate were combined, the flask was flushed with nitrogen and stoppered, and the sample was held overnight at -5°C.

The next day, the diethyl ether extract was evaporated just to dryness under vacuum using a Rinco rotating evaporator while the flask was held in a warm water bath. The lipid was immediately dissolved in petroleum ether and the extract was quantitatively transferred to a 15-milliliter conical centrifuge tube. After the chloroform-methanol extract was filtered and the solvent was evaporated according to the procedure described for serum, the petroleum ether extracts were combined. The weight of the total lipid in the red cell sample was obtained in a manner identical to that described for serum.

White Cell Total Lipids and Platelet Total Lipids

The procedures for extracting the lipids from white cells and platelets were the same. The frozen cell samples were warmed to 37°C in a water bath for 30 minutes, cooled to room temperature, and mixed well. The cell samples were added dropwise to 20 volumes of chloroform-methanol (2:1, v/v) in a beaker while the solvent was kept in motion by use of a magnetic stirrer. The lipid

extract was filtered through fat-free filter paper into an Erlenmeyer flask. The precipitate was rinsed from the filter paper into the beaker containing 20 volumes of fresh solvent. The mixture was heated to boiling and cooled. The cooled extract was filtered into the flask containing the original extract. Hot extractions of cell precipitates were repeated three times and the filtrates were combined with the original extract. Twenty percent by volume of redistilled water was added to the extract, and the water and extract were mixed thoroughly. The flask was stoppered and the mixture was allowed to stand until two layers separated and became clear. The water layer was removed and discarded, the solvent was dried, and the sample was stored according to the procedures outlined for serum.

The filter paper plus the precipitate was placed in an extraction thimble in the Soxhlet apparatus and was refluxed with 20 ml diethyl ether for a total of 15 to 20 hours in a nitrogen atmosphere. The solvent was changed after two and four hours, and then the sample was refluxed overnight. The three diethyl ether extracts were combined.

The next day, the diethyl ether was evaporated and the lipid was dissolved in petroleum ether and quantitatively transferred to a 15-milliliter conical centrifuge tube as described in the procedure for red cells. The chloroform-methanol extract was filtered, the solvent was evaporated, and the lipid sample was dissolved in

petroleum ether and quantitatively transferred to the tube containing the diethyl ether extract. The weight of the total lipid in the cell sample was obtained in a manner identical to that described for serum.

Quantitation of Total Lipid Extracts

Methodology Studies

Quantitative analysis by thin-layer chromatography was investigated as a means of determining the distribution of lipid classes in total lipid extracts. Purdy and Truter (78, 79) reported that the amount of material on a thin film chromatogram could be determined from the size of the spot it formed, irrespective of the intensity of the color. However, several attempts to verify the weight-area relationship for lipid classes were not successful.

Gravimetric measurements of the lipid classes isolated by preparative thin-layer chromatography also were investigated but were not successful. The adsorbent contaminating the samples could not be removed sufficiently for accurate weighings of the micro amounts of lipids available.

When quantification by thin-layer chromatography and gravimetric analyses proved to be unsatisfactory, the distribution of lipid classes was quantitated by direct chemical analyses of the total lipid extract for free and total cholesterol and lipid phosphorus

and by subsequent calculations. In addition to the chemical analyses of the total lipid extracts, a thin-layer chromatogram of each of the samples was prepared and photographed which allowed for some semi-quantitative visual evaluation.

Method

Procedures for the determination of free and total cholesterol and lipid phosphorus in blood fractions are described in detail in a manuscript previously prepared by the author (86). The only modification in the procedure was an adjustment to the analysis of total lipid extract instead of analysis of the blood sample. Approximately 25 μg of total lipid extract was analyzed for total cholesterol in serum and red cells and free cholesterol in red cells, and about 50 μg, for free cholesterol in serum. Five micrograms of total lipid was analyzed for both free and total cholesterol in white cells and platelets. The lipid phosphorus concentration was determined on aliquots of lipid extract containing 5 to 8 μg of total lipid.

The amounts of cholesterol esters were calculated as cholesterol oleate by multiplying the grams of esterified cholesterol by 1.68. The phospholipids were calculated by multiplying the grams of lipid phosphorus by 25. The percentages of cholesterol esters, free cholesterol, and phospholipids were computed and the value of triglycerides plus free fatty acids were calculated by difference.

It is recognized that the value for triglycerides plus free fatty acids also included the weight of the unidentified factor which appeared at the solvent front of the thin-layer chromatograms.

Separation of Total Lipids into Lipid Classes

Methodology Studies

Two methods for the separation of total lipid mixtures into classes were investigated: separation by column chromatography and by thin-layer chromatography. Although successful separations of the major classes of lipids have been reported using both macro (45) and micro (56) silicic acid columns, several problems were encountered in separating the micro amounts of lipids available from the four blood fractions. Separation and the reproducibility of the results were affected not only by the way in which the silicic acid column was packed, the temperature of the column, and the rate of flow and the volume of eluting solvent, but also by variations in composition as well as the quantity of lipid sample.

Separation of lipid classes by thin-layer chromatography was successful and this method was used. The merits of this method were stated by Randerath (80) and Mangold (63) to be rapidity and reproducibility. The one disadvantage was the larger surface of lipid that was exposed to air.

The procedures for separating the total lipid sample by

preparative thin-layer chromatography were described by Maier and Mangold (60) and in Brinkmann Instruments' Bulletin No. 22 (18). Although good resolution of all the lipid classes was obtained when this method was used, further thin-layer chromatographic analysis of each of the components separated from the complex mixture showed retention and tailing of the neighboring lipid classes (Plate 5A). To further purify the lipid classes, the individual lipid components were re-chromatographed by the thin-layer procedure and isolated a second time (Plate 5B).

Method

Equipment

1. Mounting board for glass plates, 22 x 113 cm, with retaining ledges 1.8 cm wide along a short and a long side.[1]
2. Desaga standard applicator, Model S-II, for mechanically producing a standard thickness.[1]
3. Glass plates of uniform thickness: 5 x 20 cm and 20 x 20 cm.[1]
4. Labeling template, graduated.[1]
5. Developing tanks.[2]

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[1] Brinkmann Instruments, Inc., 115 Cutter Mill Road, Great Neck, Long Island, New York.

[2] Arthur H. Thomas, Co., Philadelphia, Pennsylvania.

Plates 5A and 5B.

Lipid classes isolated by preparative thin-layer chromatography of total lipid of serum.

A. Thin-layer chromatogram following the first isolation.

B. Thin-layer chromatogram following purification by re-chromatographing the individual classes.

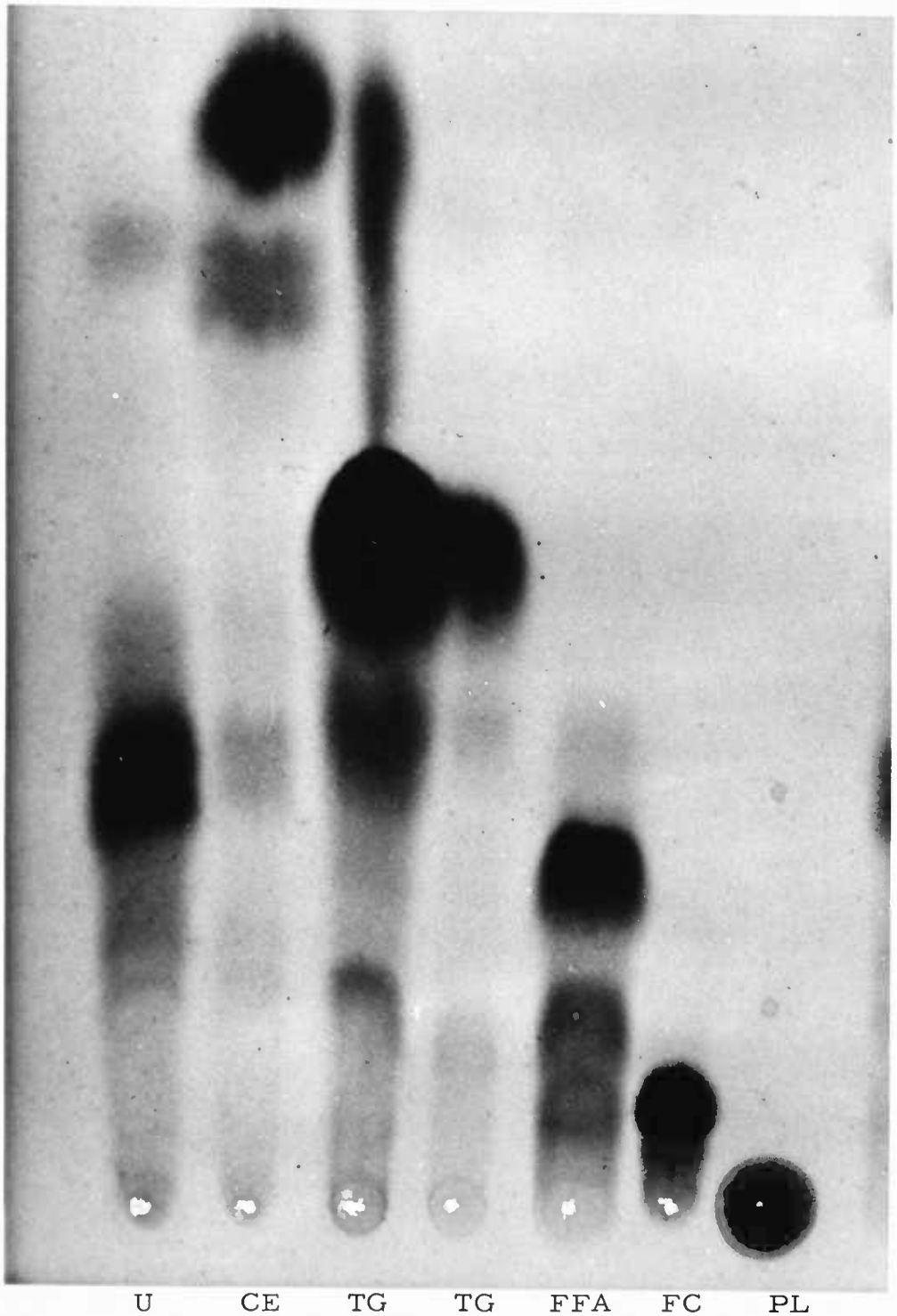

Plate 5B.

6. Racks for glass plates: metal and wooden.
7. Oven, 110°C.
8. Hamilton microliter syringes: 10, 50, 100 µl.
9. Hot plate.
10. Heating rack for thin-layer chromatographic plates.
11. Spray bottle.
12. Filter paper, Whatman No. 1, 45 x 60 cm sheets.
13. Pouring funnels.
14. Erlenmeyer flasks, 50 ml.
15. Sintered glass filters, C, 15 and 30 ml capacity.
16. Tubes: 20 x 150 mm, 12 x 100 mm.
17. Razor blades, single edge.

Reagents

1. Silica Gel H.[1]
2. Indicator, saturated chromic-sulfuric acid solution.
3. Iodine.
4. Acetone, reagent grade.
5. Glacial acetic acid.
6. Skelly Solve B (petroleum ether), distilled and the fraction distilling between 66-69°C was collected.

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[1] Brinkman Instruments, Inc., 115 Cutter Mill Road, Great Neck, Long Island, New York.

7. Diethyl ether, Mallinckrodt's purified for fat extraction. Diethyl ether was stored in the cold.
8. Developing solvent: petroleum ether-diethyl ether-glacial acetic acid (80/20/1, v/v/v).
9. Methanol, absolute. Methanol was distilled over potassium hydroxide.
10. Chloroform, reagent grade.
11. Nitrogen gas.
12. Lipid standards for thin-layer chromatography.[1]

Procedure

The procedures for coating glass plates with adsorbent, applying the sample, and eluting the lipid classes were outlined by several investigators (18, 60). The glass plates were thoroughly cleaned with a cleaning agent, rinsed well with water, and air dried. Just before use the plates were further cleaned by washing with acetone to remove any dust or contaminating materials. Silica Gel H was used as the adsorbent for coating the glass plates, and the volume of slurry required to coat five 20 x 20 cm plates contained 27 gm Silica Gel H and 72 ml redistilled water. The coated glass plates were activated by heating in an oven at 110°C for two hours. Prior to separating the lipid components, the coated plates were developed

[1] The Hormel Institute, Austin, Minnesota.

with chloroform for further activation of the adsorbent and to remove impurities. The sample of total lipid, dissolved in petroleum ether, was applied to the layer with a micro pipet at a point approximately two centimeters from the edge of the plate at right angles to the direction in which the plates were coated. A lipid standard prepared for thin-layer chromatography was applied to the layer as a marker for identifying the lipid classes. Immediately after the sample was applied, the plate was placed in a developing tank which previously had been saturated by adding 100 ml of developing solvent. Saturation was accomplished by lining the tank with a filter paper wick.

When the solvent front had reached a pre-determined mark, the plate was removed from the chamber, air dried, and quickly placed in an iodine atmosphere just until the lipid components became visible (yellow spots). The plate was removed from the iodine atmosphere, the areas of particular lipid classes were located, and the portion of Silica Gel H containing the respective lipid component was immediately scraped off into an Erlenmeyer flask containing eluting solvent. Diethyl ether was used for the elution of cholesterol esters, triglycerides, and free fatty acids. Phospholipids were eluted from the adsorbent with methanol. The Silica Gel H-lipid mixture was filtered through a sintered glass filter into a 20 x 150 mm tube. The flask was rinsed several times with solvent to

insure quantitative transfer of the lipid. The solvent was evaporated just to dryness in a nitrogen atmosphere while the tube was held in a warm water bath. The lipid was dissolved in petroleum ether and quantitatively transferred to a 12 x 100 mm tube, and the volume of solvent was concentrated to less than 0.5 ml. The individual lipid components were re-chromatographed on activated and washed plates, developed, isolated, and eluted from the Silica Gel H as previously described. The sample was filtered and the solvent was evaporated. The lipid was dissolved in petroleum ether and quantitatively transferred to a 12 x 100 mm tube which was stoppered with a cork stopper, and the sample was held at -5°C until the time of preparation for gas-liquid chromatographic analysis.

Gas-Liquid Chromatographic Analysis of Methyl Esters of Fatty Acids

Methodology Studies

The methyl esters of fatty acids of cholesterol esters, triglycerides, and phospholipids were prepared by interesterification using a modification of the method of Stoffel, Chu and Ahrens (89). According to the procedure of Stoffel et al., the lipid samples were refluxed for two hours to methylate the fatty acids. Thin-layer chromatographic analysis of the samples showed that the fatty acids of the triglycerides were completely methylated, but that those of the

cholesterol esters and phospholipids were only partially methylated. For complete methylation of the cholesterol ester and phospholipid fatty acids, the refluxing times were increased to six hours. The methyl esters were purified only by evaporation of the reagents and drying. Further purification by washing as recommended by Stoffel et al. or by thin-layer chromatographic isolation (95) were not feasible for these small quantities. The probable presence of some substances other than methyl esters was apparent by the resolution of short peaks which appeared early in the gas-liquid chromatographic record, but these were only a minor percentage of the total.

Method

Equipment

1. Microinteresterification assembly: microsublimation tubes, T joint 24/25, Soxhlet condensers, six-place manifold with nitrogen inlet and an aluminum heating block.
2. Thermometer, 150-200°C.
3. Pipet filler.
4. Micro porous boiling chips.
5. Tubes, 12 x 100 mm.
6. Conical centrifuge tubes, 4 ml.
7. Barber-Coleman gas chromatograph with thermal conductivity and hydrogen flame detectors.[1]

[1] Barber-Coleman Company, Rockford, Illinois.

8. Glass column, 8 feet, 4 mm I. D.,[1] packed with 15 percent diethylene glycolsuccinate by weight on 80/100 mesh Chromosorb W.[1]

9. Hamilton microliter syringes: 1, 10 μl.

Reagents

1. Methanol, absolute. Methanol was distilled over potassium hydroxide.

2. Hydrochloric acid in superdry methanol, 5 percent solution. A weighed amount of hydrochloric acid gas was bubbled into a weighed amount of methanol.

3. Benzene, distilled.

4. Skelly Solve B (petroleum ether), distilled and the fraction distilling between 66-69°C was collected.

5. Chloroform, distilled. Five-tenths to one percent ethanol was added as a preservative. Chloroform was stored in the cold.

6. Nitrogen, helium, and hydrogen gases and compressed air.

7. Sodium sulfate, anhydrous.

8. Lipid standards for gas-liquid chromatography.[2]

Procedure

The lipid samples to be methylated, 0.1 to 1 mg, were

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[1] Applied Science Laboratories, Inc., State College, Pennsylvania.
[2] The Hormel Institute, Austin, Minnesota.

dissolved in a mixture of 4 ml of five percent hydrochloric acid in superdry methanol and 2 ml of dry benzene in a microsublimation tube to which a condenser was connected. Boiling chips were used to prevent bumping of the solvents. The tube was placed in a heating block and the mixture was refluxed at 90-100°C in a nitrogen atmosphere for two hours for the interesterification of triglycerides and six hours for the interesterification of cholesterol esters and phospholipids. The samples were shaken frequently at the start of the refluxing period to dissolve the lipid mixture. At the end of the refluxing, the hydrochloric acid, methanol, and benzene were evaporated under a stream of nitrogen while the tube was held in a warm water bath. To remove any water in the sample, a small amount of benzene was added which was then blown off with nitrogen. Drying the sample with benzene was repeated twice if necessary. The esters were dissolved in a small volume of petroleum ether and quantitatively transferred to a 12 x 100 mm tube. The sample was further dried with anhydrous sodium sulfate and the methyl ester sample was quantitatively transferred to a four-milliliter conical centrifuge tube for ease in concentrating the sample in the bottom of the tube. The solvent was evaporated in preparation for injection of the sample on the gas-liquid chromatographic column. The methyl ester sample was dissolved in a few microliters of chloroform and injected into the column using a microliter syringe. The temperature of

the column was 180-185°C, the temperature of the flash heater was 220-225°C, and the temperature of the cell block was 200-205°C. The rate of flow of the carrier gas was adjusted to give good resolution of the methyl esters of fatty acids. A thermal detector was used to measure the methyl esters of the lipid classes in serum and of phospholipids in red cells. A hydrogen detector was used for all other samples. The proportional composition of the fatty acids in the total sample was obtained by a triangulation of the area associated with each component and calculation of the percentage of the total area.

Standard mixtures of methyl esters of fatty acids were analyzed at frequent intervals for identification of the various fatty esters and to determine the degree of quantitative resolution obtained with the specific column and instrument in use. The amounts of fatty acids calculated as components of several standard mixtures varied no more than $\pm$ 5 percent of the actual values.

EXPERIMENTAL PROCEDURE

Description of Subjects

Nine women and seven men who were active professionally or as homemakers served as experimental subjects. One person in the group, ES, served as an experimental subject for nine days in a four-week period. The ages ranged from 23 to 66 years. Data on their sex, age, height, and weight are presented in Table 8. The subjects are grouped as female and male and in ascending order of age.

Table 8. Data on Sex, Age, Height, and Weight of Sixteen Subjects

Subject	Sex	Age	Height cm	Weight kg
KG	F	23	159	46
MM[1]	F	26	177	64
SP	F	28	163	51
ES	F	28	157	56
DB	F	42	157	70
BH[2]	F	42	175	70
EJ[2]	F	46	157	62
MB[2]	F	63	165	74
MF[2]	F	63	165	69
CG	M	27	185	75
RM	M	33	184	75
KP[2]	M	33	183	86
JJ[2]	M	40	168	72
SB	M	41	178	78
HH[2]	M	56	183	82
EB[2]	M	66	173	79

[1] Lactating since November 8, 1963.
[2] Minor organic disorders discussed in text.

Although the subjects were in generally good health, organic disorders were reported by five persons. Subject EJ had chronic glomerular nephritis which was under control at the time the blood sample was taken. The use of salt was limited in her diet. Subjects MF and JJ reported allergies. Subject MF had a skin allergy which was controlled by applying sulfur ointment, by treatment with ultra violet rays and X-rays, and by limiting the use of nuts, chocolate, and hot tea and coffee in her diet. Subject JJ received allergy shots. Subject MB had an underactive thyroid and took thyroid tablets daily. Subject EB used pain capsules to relieve his arthritic condition.

Values for red and white cell counts and differential counts were within the normal ranges cited by Wintrobe (106) and are presented in Appendix I.

The general diet pattern of each subject was assessed using a questionnaire. The questionnaire was similar to the diet history record used in the Cooperative Nutritional Status Studies in the Western Region (103) but modified to obtain more detailed estimates of the kinds and amounts of fat consumed (Appendix II). The questionnaire was scored according to the basic diet pattern recommended to meet the nutritional needs of healthy individuals (105). In general, the dietary intake of the subjects was good and the fat intakes were estimated to be moderate (Appendix III A). Several subjects reported that they used more vegetable fat than formerly and more oil than

solid fat. A one-day diet record also was kept by each subject for the day preceding the blood sampling. The record was evaluated and scored, and the total calories, protein, carbohydrate, and fat were calculated. The percentage of fat calories for the sixteen subjects ranged from 32 to 52 percent. When the total fat was calculated as saturated and unsaturated fatty acids, it was found that the subjects had an unsaturated fatty acid intake which was equal to or greater than the saturated fatty acid intake. The proportion of polyunsaturated fat to saturated ranged mainly from 0.2 to 0.5 (Appendix III A). The percentage of fat calories for the one subject, whose blood was analyzed on nine different days, ranged from 29 to 47 percent with an average value of 40 percent (Appendix III B).

Blood Analysis

Blood was taken from the antecubital vein of subjects who had been fasting overnight for at least 12 hours. About 30 ml of blood was drawn for serum, red cell, white cell, and platelet separation, cell counts, and differential counts. The enumeration of red and white cell counts[1] and differential counts[2] was by standard clinical procedures. Blood fractions were isolated according to the

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[1] The enumeration of red and white cells by S. Park.

[2] Differential cell counts by technicians at the Good Samaritan Hospital Clinical Laboratory, Corvallis, Oregon.

procedures on pages 19 to 23. The size of the samples isolated for analysis were about 1 ml each of serum and packed red cells and about 30 mg wet weight each of white cells and platelets. The methods for extracting the total lipid from blood fractions are described on pages 34 to 40. The quantitation of the lipid extracts was done according to the procedures discussed on pages 41 to 42. The separation of total lipid extracts into lipid classes and the determination of methyl esters of fatty acids are described on pages 43 to 50 and 51 to 54, respectively.

RESULTS AND DISCUSSION

Total Lipids

The concentrations of total lipid in the blood fractions of the sixteen subjects are presented in Table 9 and for the nine samples of the one subject in Table 10. The amounts of total lipid in sera and in red cells were similar, whereas white cell and platelet samples contained two to four times as much. The range of values for lipids in the blood fractions were: sera, 455 to 913 mg per 100 ml; red cells, 476 to 663 mg per 100 ml; white cells, 2381 to 3800 mg per 100 gm; and platelets, 1959 to 3131 mg per 100 gm. For serum, all the values except one, 913 mg per 100 ml for subject EB, were within the normal range cited by Albritton (2). Although plasma, instead of serum, was analyzed for subject EB, serum and plasma have been reported to have comparable total lipid concentrations (2). Total lipids determined in red cells, likewise, were within the normal range cited by Albritton (2), whereas the concentrations in white cells were higher than the few values which have been reported for human subjects (9, 15). Total lipids in platelets were comparable to or slightly lower than those in white cells. These values found in this study could not be compared to those reported by others, since platelet lipids have been reported only as percentages of the dry weight.

Table 9. Total Lipids and the Distribution of Lipid Classes in the Blood Fractions of Sixteen Subjects

Blood Fraction	Subject	Total Lipid mg/100 ml	Cholesterol Esters	Free Cholesterol	Triglycerides Plus Free Fatty Acids	Phospholipids
			-- Percent Total Lipid --			
Serum	KG	455	33	8	27	32
	MM	513	42	7	21	30
	SP	635	34	8	29	29
	ES	547	36	8	28	28
	DB	528	36	8	25	31
	BH	619	38	8	26	28
	EJ	588	35	8	25	32
	MB	679	37	8	25	30
	MF	517	35	8	27	30
	CG	614	35	8	23	34
	RM	521	39	10	15	36
	KP	603	34	8	22	36
	JJ	727	42	7	22	29
	SB	504	39	8	22	31
	HH	689	40	8	17	35
	EB[1]	913	34	8	28	30
	Mean	603 ± 28.0[2]	37 ± 0.8	8 ± 0.3	24 ± 1.0	31 ± 0.8
Red Cell	KG	476	0	29	6	65
	MM	501	0	25	12	63
	SP	518	0	24	17	59
	ES	546	0	23	16	61
	DB	663	0	20	12	68
	BH	620	5	22	11	62
	EJ	546	1	23	16	60
	MB	586	0	23	14	63
	MF	542	2	21	17	60
	CG	555	0	21	24	55
	RM	518	0	27	10	63
	KP	528	0	23	25	52
	JJ	614	3	19	17	61
	SB	505	0	28	5	67
	HH	652	0	21	14	65
	EB	517	2	24	17	57
	Mean	555 ± 14.0	1 ± 0.3	23 ± 0.8	15 ± 1.3	61 ± 1.0

[1] Plasma analyzed instead of serum.

[2] Standard error of the mean (87).

Table 9. (Continued)

Blood Fraction	Subject	Total Lipid mg/100 gm	Cholesterol Esters	Free Cholesterol	Triglycerides Plus Free Fatty Acids	Phospholipids
			-- Percent Total Lipid --			
White Cell	KG	2381	5	16	13	66
	MM	2484	7	23	3	67
	SP	2382	3	9	17	71
	ES	3552	11	11	6	72
	DB	2416	4	14	17	65
	BH	2987	13	12	13	62
	EJ	3420	11	20	5	64
	MB	3352	10	19	4	67
	MF	3190	5	14	13	68
	CG	2866	9	20	4	67
	RM	2614	8	22	7	63
	KP	3146	4	12	12	72
	JJ	3800	8	16	3	73
	SB	3637	4	24	7	65
	HH	3533	4	14	16	66
	EB[1]					
	Mean	3051 ± 128.2[2]	7 ± 0.8	16 ± 1.3	9 ± 1.3	67 ± 0.8
Platelet	KG	1959	8	23	3	66
	MM	2373	6	17	9	68
	SP	2097	4	20	11	65
	ES	3019	9	17	11	63
	DB	2722	6	29	3	62
	BH	2673	8	19	9	64
	EJ	2821	5	21	11	63
	MB	2914	11	22	4	63
	MF	2565	9	24	3	64
	CG	2288	8	26	4	62
	RM	2725	3	14	19	64
	KP	2096	8	26	4	62
	JJ	2843	7	22	5	66
	SB	2585	7	21	7	65
	HH	3131	6	16	9	69
	EB[3]					
	Mean	2587 ± 91.0	7 ± 0.5	21 ± 1.0	7 ± 1.0	64 ± 0.5

[1] No white cell sample.

[2] Standard error of the mean (87).

[3] No platelet sample.

Table 10. Total Lipids and the Distribution of Lipid Classes in the Blood Fractions of One Subject on Nine Different Days

Blood Fraction	Day	Total Lipid mg/100 ml, mg/100 gm.	Cholesterol Esters	Free Cholesterol	Triglycerides Plus Free Fatty Acids	Phospholipids
			-- Percent Total Lipid --			
Serum	1	547	36	8	28	28
	2	538	48	11	9	32
	3	583	38	8	26	28
	4	521	36	8	26	30
	5	529	37	8	25	30
	10	582	36	8	25	31
	18	552	37	7	28	28
	25	540	37	8	26	29
	31	595	35	8	27	30
	Mean	554 ±8.7[1]	38 ±1.3	8 ±0.3	24 ± 2.0	30 ± 0.3
Red Cell	1	546	0	23	16	61
	2	538	0	23	12	65
	3	559	2	21	15	62
	4	539	0	23	15	62
	5	528	0	25	11	64
	10	535	0	25	12	63
	18	506	0	27	9	64
	25	522	0	29	9	62
	31	554	0	25	8	67
	Mean	536 ± 5.3	0.2 ± 0.2	25 ± 0.7	12 ± 1.0	63 ± 0.7
White Cell	1	3552	11	11	6	72
	2	3656	4	18	14	64
	3	2957	11	18	5	66
	4	2828	11	14	5	70
	5	3163	9	15	8	68
	10	2780	7	19	6	68
	18	3030	10	13	10	67
	25	3727	9	14	9	68
	31	2530	11	18	8	63
	Mean	3136 ± 140.7	9 ± 0.7	16 ± 1.0	8 ± 1.0	67 ± 1.0
Platelet	1	3019	9	17	11	63
	2	2993	7	15	18	60
	3[2]					
	4	2596	8	19	7	66
	5	2361	6	14	17	63
	10	2734	4	13	16	67
	18	3368	7	18	4	71
	25	2631	7	15	9	69
	31	2776	8	14	13	65
	Mean	2810 ± 111.1	7 ± 0.4	16 ± 0.7	12 ± 1.8	66 ± 1.4

[1] Standard error of the mean (87).
[2] No platelet sample.

Total lipids in all of the blood fractions varied not only from person to person but also from day to day for the one subject. Values in sera and red cells showed less day-to-day variation for the one subject than among the sixteen, which would be expected (27, 30). The variations in white cells and platelets for the one subject, however, were equal to or slightly greater than those among the whole group.

It is recognized that part of the variation in the total lipids of white cells and platelets is due to difficulties inherent in the measurement of wet weights of these cells. In one series of analyses of replicate aliquots of white cells and of platelets, total lipids determined for single aliquots ranged up to 20 percent of the mean value for white cells and 30 percent, for platelets. Total lipids determined for most aliquots, however, showed less variation than these maxima from the means.

Total lipids in the four blood fractions of each subject were compared with the respective average values to determine whether a high concentration of lipid in one fraction was associated with corresponding high values in all others, or whether a low value in one was associated with low values in all others. Only six of the sixteen subjects exhibited this pattern, subjects KG, MM, MB, CG, JJ, and HH. Subject KG, for example, had the lowest concentration in all four fractions. Similarly, no consistent day- to-day pattern

was exhibited among the blood fractions of the one subject. It was concluded from this limited amount of data that total lipid in one blood fraction does not indicate whether the concentrations in other fractions will be high or low.

Distribution of Lipid Classes

The distribution of lipid classes found in the blood fractions of the sixteen subjects, Table 9, varied from person to person but certain characteristic patterns were apparent. These are illustrated in Plate 6. Serum was characterized by high cholesterol ester and triglyceride concentrations, together with phospholipids. In red cells, phospholipids and free cholesterol comprised most of the total lipid, whereas there were generally lower amounts of triglycerides than in serum and almost no cholesterol esters. Also, in red cells there was a relatively high concentration of an unidentified component which, in Tables 9 and 10, was included in the triglyceride plus free fatty acid value. Lipid classes in white cells and platelets were similar. Both contained high concentrations of phospholipids, considerable amounts of free cholesterol, some cholesterol esters, as well as some of the unidentified component, and a relatively high proportion of free fatty acids.

The mean values (and ranges) of the lipid classes in serum for the sixteen subjects were: cholesterol esters, 37 percent (33 to 42);

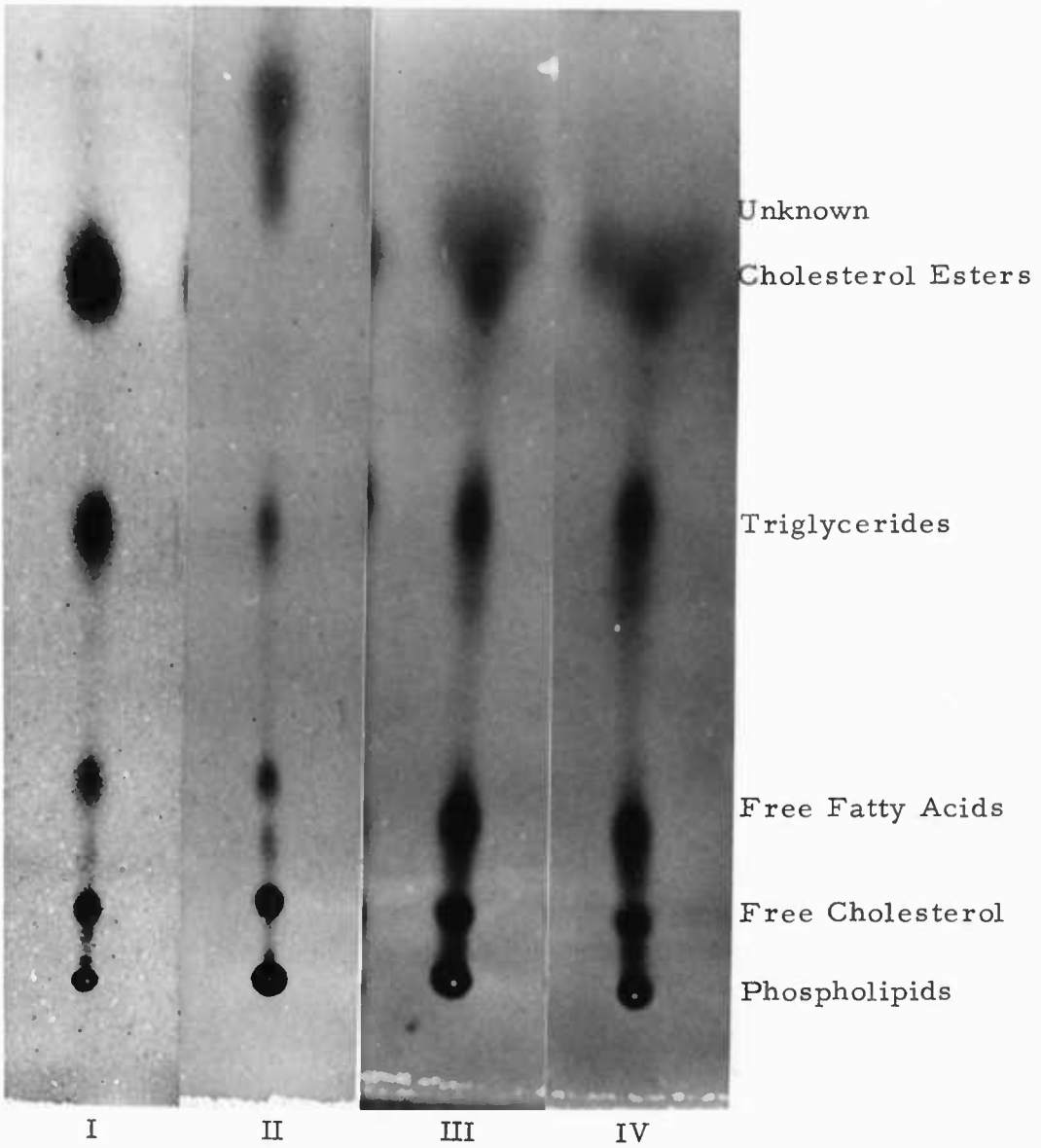

Plate 6. Distribution of lipid classes in total lipid extracts of the four blood fractions: I, serum; II, red cells; III, white cells; IV, platelets.

free cholesterol, 8 percent (7 to 10); triglycerides plus free fatty acids, 24 percent (15 to 29); and phospholipids, 31 percent (28 to 36). These values were quite similar to those reported by others (Table 1) except that the triglycerides plus free fatty acids in this study averaged nearly one-fourth of the total lipid as compared to the one-fifth found by others. The mean values (and ranges) of lipid components in red cells were: cholesterol esters, 0 to 5 percent; free cholesterol, 23 percent (19 to 29); triglycerides plus free fatty acids, 15 percent (5 to 25); and phospholipids, 61 percent (52 to 68). The distribution of lipid classes in red cells showed good agreement with values reported in the literature (Table 3). Although the few studies which reported the distribution of lipid classes in white cells showed wide variation (Table 5), the values obtained in this study fit into the same general pattern. The mean percentages (and ranges) of lipid classes in white cell lipids were: cholesterol esters, 7 (3 to 13); free cholesterol, 16 (9 to 24); triglycerides plus free fatty acids, 9 (3 to 17); and phospholipids, 67 (62 to 73). The percentages of the lipid classes in the total lipid extracts of platelets of sixteen subjects differed from the pattern described by other investigators (Table 7) in that they contained smaller amounts of phospholipids but more free cholesterol and triglycerides plus free fatty acids. The mean values (and ranges) of lipid classes were: cholesterol esters, 7 percent (3 to 11); free cholesterol, 21 percent (14 to 29); triglycerides

plus free fatty acids, 7 percent (3 to 19); and phospholipids, 64 percent (62 to 69).

The day-to-day variations of lipid classes in blood fractions of the one subject and the variations from person to person among the sixteen subjects were about the same, as evaluated by a comparison of the standard errors of the means. In Tables 9 and 10, it is apparent that all of the lipid classes in serum and the phospholipids in all formed elements showed the least variation. Triglycerides plus free fatty acids in formed elements and cholesterol esters in white cells and platelets showed the greatest variations. The greater variability of the triglycerides plus free fatty acids could be attributed, at least in part, to the method of calculating the values by difference. Semi-quantitative evaluation of photographs of thin-layer chromatograms, which were prepared, did not confirm the degree of variation that was found in the calculated values. The variations found in the cholesterol esters of white cells and platelets, which were proportionally greater than that in serum, may be related to the role of these cells in the synthesis of lipids.

Within the limited degree of variation found in this study, there was no consistent relationship between the concentrations of different lipid classes or of total lipid and the age or sex of the subjects. Similarly, there was no relationship found between the lipid values and fat intake as evaluated by the diet questionnaire and the

one-day diet record, either among the sixteen subjects or day to day for the one subject.

The ratios of free to total cholesterol in the blood fractions of sixteen subjects are presented in Table 11 and for nine samples of the one subject, in Table 12. The proportions of free cholesterol in sera agreed with values reported in the literature and were about one-fourth of the total cholesterol. That the cholesterol in red cells was mainly present in the free form was confirmed. As in other studies (4, 74, 86), greater amounts of free than total cholesterol were determined for several subjects for which there is no explanation. In contrast to earlier findings in this laboratory (86) in which the proportions of free to total cholesterol in white cell-platelet samples varied from 33 to 97 percent, the proportions of free cholesterol in white cells and in platelets showed less variability from person to person and values for white cells and platelets ranged only from 63 to 91 percent and 76 to 90 percent, respectively. Concentrations of total cholesterol, calculated on the basis of mg per 100 gm sample, determined in this study also were lower than those found in the earlier study (86). These differences may be accounted for by the methods of estimating sample sizes in the two studies, i. e, direct weighing of wet weights in this study and an indirect estimate based on the amount of total phosphorus analyzed in the previous study.

Table 11. Percentages of Free to Total Cholesterol in the Blood Fractions of Sixteen Subjects

Subject	Serum	Red Cell	White Cell	Platelet
KG	29	108	85	83
MM	21	107	84	83
SP	28	118	85	90
ES	26	102	63	77
DB	26	102	86	90
BH	26	88	64	81
EJ	28	98	76	88
MB	26	105	75	78
MF	29	94	84	83
CG	29	107	80	84
RM	30	101	83	89
KP	28	113	84	84
JJ	22	92	77	85
SB	24	111	91	84
HH	25	114	85	82
EB[1]	29	95		

[1] No white cell or platelet samples.

Table 12. Percentages of Free to Total Cholesterol in the Blood Fractions of One Subject on Nine Different Days

Day	Serum	Red Cell	White Cell	Platelet
1	26	102	63	77
2	27	104	88	77
3[1]	25	95	76	
4	27	117	68	81
5	27	116	74	80
10	26	102	81	85
18	25	108	68	80
25	27	104	73	78
31	28	109	74	76

[1] No platelet sample.

Fatty Acid Composition of Lipid Classes

The percentages of fatty acids combined in cholesterol esters, triglycerides, and phospholipids in the four blood fractions for sixteen subjects are presented in Tables 13, 14, and 15, respectively. The mean values and the standard errors of the means for all classes are presented in Table 16. The values are expressed as percentages of the total fatty acids which were resolved by gas-liquid chromatography. Some minor components are not tabulated. In red cell samples, cholesterol esters were present in too small an amount to isolate adequately for accurate analysis and the compositions of the free fatty acid class in the four fractions of the blood were not analyzed. The commonly accepted abbreviations of fatty acids were used in the tables: lauric, 12:0; myristic, 14:0; palmitic, 16:0; palmitoleic, 16:1; stearic, 18:0; oleic, 18:1; linoleic, 18:2; linolenic, 18:3; arachidic, 20:0; eicosatrienoic, 20:3; and arachidonic, 20:4. One acid, which appeared after palmitoleic and before stearic acid on the gas-liquid chromatographic record, was not identified and it was designated in the tables as ?. The designation of > 20:4 referred to the total of one or more acids appearing after arachidonic acid which were not identified.

The relative proportions of fatty acids in cholesterol esters differed in white cells and platelets from those found in serum

Table 13. Percentages of Fatty Acids of Cholesterol Esters in the Blood Fractions of Sixteen Subjects

Blood Fraction	Subject	Fatty Acids												
		12:0	14:0	16:0	16:1	?	18:0	18:1	18:2	18:3	20:0	20:3	20:4	>20:4
Serum	KG	1	1	20	6		8	18	44				tr	
	MM	tr	tr	17	6	1	3	18	47		tr	2	4	
	SP	tr	1	19	6	1	3	17	48	tr	tr		4	
	ES	tr		16	5	tr	3	19	55					
	DB	1	tr	18	4	tr	3	18	48				7	
	BH	tr	tr	16	5		3	20	47				6	
	EJ	tr	tr	25	9	2	5	22	36					
	MB		2	16	3	1	5	22	45				3	
	MF	1	17	16	5	tr	1	16	37				4	
	CG	tr	tr	20	5	2	6	17	44		tr		3	
	RM	tr	tr	17	5	2	3	22	47				3	
	KP	tr	6	18	5		2	18	46					
	JJ	1	17	13	6	1	1	17	37				4	
	SB	tr	9	14	6	4	2	25	37				2	
	HH	tr	2	16	5		4	20	51	tr			3	
	EB[1]	tr	2	19	3	4	7	19	39	2			3	
White Cell	KG	1	2	25	6	4	15	17	8	tr	tr	3		8
	MM	2	1	20	6	5	14	16	12	8	tr	5		5
	SP	1	tr	24	10	19	7	15	4	tr	1			7
	ES	1	tr	30	8	9	13	19	4	tr	tr	2	tr	3
	DB	1	1	35	11	7	13	13	5	1	1			8
	BH	1		30	8	8	15	16	7	tr	tr	2		2
	EJ	tr	tr	29	7	13	13	16	2	3	1		tr	3
	MB	2	1	30	12	5	11	11	4	tr	tr	2		10
	MF	1	1	20	9	8	11	16	5		10	3		4
	CG	tr	2	34	11	3	12	18	3	tr	tr	tr		8
	RM	2	tr	26	12	7	10	15	5	tr	1	3	tr	6
	KP	1		32	10	4	10	14	3		4			16
	JJ	1		23	11	6	11	20	6	tr	1	3		2
	SB	1	2	27	12	12	11	13	6	tr	tr	tr		6
	HH	1	tr	24	14	16	10	14	4	1	1			7
	EB[2]													

[1] Plasma analyzed instead of serum.

[2] No white cell sample.

Table 13.(Continued)

Blood Fraction	Subject	Fatty Acids												
		12:0	14:0	16:0	16:1	?	18:0	18:1	18:2	18:3	20:0	20:3	20:4	>20:4
Platelet	KG	1	tr	30	10	3	9	13	5	1	4			6
	MM	2	2	27	8	6	14	16	5	2	2	2		3
	SP	1	tr	26	8	5	12	19	6	1	1	tr	tr	12
	ES	2	1	30	8	4	13	17	4	tr	tr	tr		4
	DB	1	tr	21	10	24	9	10	7	tr	tr	4		8
	BH	1	tr	21	7	9	9	16	3	2	2	5	4	3
	EJ	2	tr	24	12	10	10	18	5	tr	tr	2	2	4
	MB	tr	1	22	20	12	8	12	7	tr	1	3	1	4
	MF	1	2	22	9	6	11	23	7	tr	tr	2		5
	CG	2	1	31	17	6	10	23	1	tr		tr	tr	tr
	RM	3	1	25	10	12	13	13	3	tr	1	1		2
	KP	1	tr	25	12	9	10	17	4	1	2	1	2	7
	JJ	1	1	27	10	2	11	26	5	1	1	2		4
	SB	1	tr	28	8	6	14	22	5					10
	HH	1	tr	21	8	19	10	14	8	tr	tr			4
	EB[1]													

[1] No platelet sample.

Table 14. Percentages of Fatty Acids of Triglycerides in the Blood Fractions of Sixteen Subjects

Blood Fraction	Subject	Fatty Acids												
		12:0	14:0	16:0	16:1	?	18:0	18:1	18:2	18:3	20:0	20:3	20:4	>20:4
Serum	KG	tr	9	28	5		5	40	11					
	MM	tr	3	28	3	tr	8	48	9					
	SP	tr	8	27	4		8	46	8					
	ES	tr	19	32	5		6	33	5					
	DB	tr	10	32	5		5	39	8					
	BH	tr	12	29	5		6	41	8					
	EJ	tr	4	31	4	tr	7	46	8					
	MB	tr	12	31	4		5	36	12					
	MF	tr	10	30	5		5	42	7					
	CG	1	9	28	5		5	42	10					
	RM	tr	3	27	4	tr	8	49	9					
	KP	tr	6	30	3		8	45	8					
	JJ	tr	17	33	4		3	34	7					
	SB	tr	10	29	5		5	40	10					
	HH	tr	4	29	4		7	46	9					
	EB[1]	tr	4	26	4	tr	8	49	9					
Red Cell	KG	2	2	24	10	2	13	28	5	2	tr		4	
	MM	1	tr	28	11	3	10	23	5	tr	tr			13
	SP	2	2	28	8	2	11	25	7					3
	ES	2	2	27	15	2	13	25	4					
	DB	1	2	30	10	1	12	26	5	3	1			tr
	BH	tr	1	30	11	3	11	21	5	tr	tr			11
	EJ	1	1	26	18	2	9	14	4	2	tr	2		11
	MB	1	8	33	5	tr	6	43	2					
	MF	tr	1	27	10	3	13	26	4					tr
	CG	1	2	26	7	2	10	29	6	2		tr	tr	6
	RM	1	2	30	9	3	11	24	6				2	7
	KP	2	4	26	8	3	12	22	4	2	1	1	tr	1
	JJ	2	2	29	13	5	10	21	4					tr
	SB	2	2	40	11	3	11	16	3			tr	tr	4
	HH		3	31	5		4	42	15					
	EB	2	1	27	11	4	11	25	5					7

[1] Plasma analyzed instead of serum.

Table 14.(Continued)

Blood Fraction	Subject	Fatty Acids												
		12:0	14:0	16:0	16:1	?	18:0	18:1	18:2	18:3	20:0	20:3	20:4	>20:4
White Cell	KG	1	1	29	11	1	11	21	3	tr	1	1	tr	13
	MM	1	tr	30	9	1	15	25	5		1		tr	6
	SP	2	1	28	8	1	14	24	4	tr	3	1		9
	ES	2	tr	26	6	4	11	11	2	5	5		2	14
	DB	1	tr	29	12	1	13	16	3	2	2			10
	BH	2	tr	27	7	3	11	17	2	tr	3	tr	tr	11
	EJ	2	tr	28	11	1	13	25	3	1	1	2		8
	MB	2	1	29	10	3	11	25	3	tr	1	1	tr	8
	MF	1	1	33	12	1	12	18	3		2	tr		9
	CG	2	tr	27	13	4	10	19	3	tr	1		2	10
	RM	2	2	27	8	5	11	16	3	1	1	1		12
	KP	2	1	27	7	3	11	18	3	tr	1	1	tr	10
	JJ	2	1	32	11	1	12	22	3	1	1			5
	SB	1	tr	26	15	2	12	16	4	tr	1	tr		10
	HH	2	1	29	11	3	10	22	4	tr	2	2		7
	EB[1]													
Platelet	KG	2	2	30	9	1	12	22	3		4			9
	MM	3	1	28	9	2	13	23	3		1	1		5
	SP	3	1	31	9	2	11	19	3	tr	1	2		8
	ES	1	1	32	9	3	12	15	4	2	2			4
	DB	2	tr	23	14	2	11	16	5	tr	1	1	tr	13
	BH	1	1	22	9	2	11	13	4	3	3		4	18
	EJ	2	tr	29	9	3	15	19	3	1	1	2		4
	MB	3	1	33	9	4	10	21	3		tr			4
	MF	2	2	31	16	1	12	15	2	tr	1	2	2	9
	CG[2]													
	RM	2	1	28	8	3	13	20	6	tr	2		3	6
	KP	1	tr	31	9	2	12	23	6	tr	2	2		8
	JJ	1	2	30	9	1	11	17	4	2	2	tr		9
	SB	3	1	28	6	2	17	21	4	tr	tr			6
	HH	2	1	27	12	3	11	17	3	tr	1	3		10
	EB[2]													

[1] No white cell sample.

[2] No platelet sample.

Table 15. Percentages of Fatty Acids of Phospholipids in the Blood Fractions of Sixteen Subjects.

Blood Fraction	Subject	Fatty Acids												
		12:0	14:0	16:0	16:1	?	18:0	18:1	18:2	18:3	20:0	20:3	20:4	>20:4
Serum	KG			31			17	12	22				9	
	MM			29			18	12	26				13	
	SP		1	36	2		12	11	23			3	12	
	ES			29	2		17	13	26			5	8	
	DB		tr	33	1		19	12	24				11	
	BH		1	30	1		17	12	21			5	14	
	EJ	2	1	32	1		16	13	27			1	9	
	MB		1	29	1	1	17	12	23			2	14	
	MF			27	tr		13	14	23	4		3	8	
	CG			32	tr		15	10	29				9	
	RM			34			14	12	27			5	8	
	KP		tr	33	1		17	10	24			3	12	
	JJ		tr	36	1		15	14	23				8	
	SB			30	1		16	12	29				11	
	HH		tr	29	tr		14	13	18	tr			7	8
	EB[1]		tr	32	1		16	16	19			1	10	
Red Cell	KG		1	30	tr		20	20	13		1	2	10	
	MM	1	1	32	1	1	18	20	12	tr	tr		12	
	SP		tr	40	1	1	19	21	10		1		8	
	ES		1	32	1	2	18	18	11				13	
	DB		tr	27			19	22	13				19	
	BH		1	27	1	3	13	17	11			4	16	7
	EJ		tr	33		3	18	19	11				15	
	MB		1	32	1	1	19	20	12			2	13	
	MF		1	33	1	1	16	17	10	12		tr	8	
	CG		tr	29	tr		18	17	12	5				15
	RM		1	30	tr		18	21	11				18	
	KP		tr	42	tr		18	22	6					3
	JJ		1	31			19	19	11			2	16	
	SB		tr	30	tr	2	15	24	14			tr	14	
	HH		1	31	tr	3	18	17	12				18	
	EB		1	26	1	4	19	17	10		2	1	17	

[1] Plasma analyzed instead of serum.

Table 15.(Continued)

Blood Fraction	Subject	Fatty Acids												
		12:0	14:0	16:0	16:1	?	18:0	18:1	18:2	18:3	20:0	20:3	20:4	>20:4
White Cell	KG	2	1	24	5	5	20	15	5		5		12	
	MM	1	1	23	3	2	19	15	6	tr	2	3	20	3
	SP	3	tr	28	3	tr	18	9	5		9	tr	9	8
	ES	2	1	27	6	5	18	17	4	tr	3	4	7	
	DB	2		33	5	7	18	12	4	tr	2	tr	7	3
	BH	1	1	29	4	5	18	15	4	tr	3	tr	10	
	EJ	2	5	25	5	4	19	16	5	tr	1	3	8	
	MB	4	6	20	6	4	12	13	7		5	2	5	7
	MF	2	tr	25	3	3	16	13	5		6	3	13	4
	CG	2	1	23	4	3	16	11	6		7	tr	9	7
	RM	1	2	20	2	4	17	13	5	5	5	5	17	tr
	KP	3	1	23	3	4	15	11	8	tr	3	4	8	
	JJ	2	1	27	3	6	18	19	4	tr	3	4	10	tr
	SB	1	tr	17	2	5	21	14	6	tr	3	tr	25	3
	HH	3	tr	27	4	2	16	11	5		6	3	9	4
	EB[1]													
Platelet	KG	3	tr	20	3	3	19	13	5		5	3	20	1
	MM	4	1	29	4	2	17	13	4		3	4	9	
	SP	3	7	24	3	4	18	15	7		tr	tr	12	tr
	ES	2	2	29	4	tr	16	15	2		4	4	8	
	DB	1		18	2	6	20	15	6	tr	2	1	24	1
	BH	2		30	5	6	18	16	5		3	1	7	
	EJ	1	3	24	2	4	25	18	5				16	
	MB	2	5	22	6	5	15	12	4		5	tr	16	3
	MF	4	1	28	2	2	25	14	4		4		13	
	CG	2	tr	23	3	4	18	11	7		5	3	13	7
	RM	2	5	27	2	5	20	17	3		3	5	7	
	KP	1	tr	20	3	4	17	12	5		3	3	6	20
	JJ	tr	tr	25	2	2	21	15	6		1	3	20	
	SB	1	tr	17	1	5	22	17	7		3	1	23	tr
	HH	1	1	20	3	4	16	17	6		3	tr	19	3
	EB[2]													

[1] No white cell sample.

[2] No platelet sample.

Table 16. Mean Percentages of Fatty Acids of Three Lipid Classes in the Blood Fractions of Sixteen Subjects

Lipid Class	Fatty Acids												
	12:0	14:0	16:0	16:1	?	18:0	18:1	18:2	18:3	20:0	20:3	20:4	>20:4
Cholesterol Esters													
Serum	0-1	4±1.5[1]	18±0.7	5±0.4	0-4	4±0.5	19±0.6	44±1.4	0-2	0-1	0-2	4±0.5	
Red Cell[2]													
White Cell	tr-2	0-2	27±1.2	10±0.6	8±1.2	12±0.6	16±0.6	5±0.6	0-8	tr-10	2±0.5	0-1	6±0.9
Platelet	tr-3	tr-2	25±0.9	11±0.9	9±1.5	11±0.5	17±1.2	5±0.4	0-2	0-4	2±0.5	0-4	5±0.8
Triglycerides													
Serum	tr-1	9±1.2	29±0.5	4±0.3	0-1	6±0.4	42±1.3	9±0.4					
Red Cell	0-2	2±0.4	29±0.9	10±0.8	3±0.3	10±0.6	26±1.9	5±0.7	0-3	0-1	0-2	0-4	5±1.3
White Cell	1-2	tr-2	29±0.5	10±0.6	2±0.4	12±0.4	20±1.1	3±0.3	0-5	2±0.3	0-2	0-2	10±0.6
Platelet	1-3	1-2	29±0.9	10±0.6	2±0.3	12±0.5	19±0.9	4±0.3	0-3	2±0.3	0-3	0-4	8±1.1
Phospholipids													
Serum	0-2	0-1	31±0.7	0-2	0-1	16±0.4	12±0.4	24±0.8	0-4		3±0.6	10±0.6	0-8
Red Cell	0-1	tr-1	32±1.1	0-1	0-4	18±0.4	19±0.6	11±0.4	0-12	0-2	0-4	14±2.3	0-15
White Cell	2±0.3	0-6	25±1.0	4±0.4	4±0.4	17±0.6	14±0.7	5±0.3	0-5	4±0.6	0-5	11±1.4	4±0.9
Platelet	2±0.3	0-7	24±1.1	3±0.4	4±0.4	19±0.8	15±0.6	5±0.4	0-1	3±0.4	0-5	14±1.6	4±2.4

[1] Standard error of the mean (87).

[2] No cholesterol ester sample.

(Tables 13 and 16). The acids which were present in the highest concentrations in serum were palmitic, oleic, and linoleic, a pattern similar to data reported in other studies (Table 2). The mean percentages (and ranges) of the major fatty acids were: palmitic, 18 (13 to 25); stearic, 4 (1 to 8); oleic, 19 (16 to 25); linoleic, 44 (36 to 55); and arachidonic, 4 (0 to 7). In addition to these, the amounts of myristic acid for several subjects were much higher than values usually reported; values ranged up to 17 percent, although the average was only 4 percent. In a study on the composition of fatty acids in cholesterol esters of red cells, Hanahan et al. (42) reported that myristic acid was present in high proportions, averaging nine percent. Although it was not possible to analyze the cholesterol esters of red cells of the subjects in this study, data were obtained for cholesterol esters isolated from a large sample of cells. In this one sample, the proportions of fatty acids were similar to those found in serum, although there was somewhat more oleic acid and somewhat less linoleic acid in the red cells. Hanahan et al. also reported the major fatty acids in cholesterol esters of red cells to be palmitic, oleic, and linoleic but they found that the proportion of linoleic acid was much greater and the proportions of palmitic and oleic were somewhat less than in serum. The pattern of fatty acids combined with cholesterol esters in white cells and platelets were similar to each other but differed from those in serum. There was little or no

myristic acid in the cells and about one and one-half times as much palmitic acid. Palmitoleic acid and the unidentified acid having a retention time between 16:1 and 18:0 averaged two to three times greater than those in serum. There was about three times more stearic acid in cells, whereas the proportions of oleic acid were comparable in cells and in serum. In contrast to serum, the cells contained a linoleic acid concentration which was only about one-tenth the value in serum. Little or no arachidonic acid was found but, instead, the unidentified longer chain acids with retention times greater than 20:4 were present and the concentrations of these were about equal to the value of arachidonic acid in serum.

The proportions of the different fatty acids in triglycerides were more similar among all blood fractions than were the proportions of fatty acids in cholesterol esters or phospholipids. Palmitic and oleic acids were present in highest concentrations in all fractions. The percentage of fatty acids in triglycerides of serum (Tables 14 and 16) showed good agreement with data reported in other studies (Table 2), except that the average concentration of myristic acid for these sixteen subjects was higher, nine percent, as compared to three percent. The mean values (and ranges) of the major fatty acids of serum triglycerides were: palmitic, 29 percent (26 to 33); stearic, 6 percent (3 to 8); oleic, 42 percent (33 to 49); linoleic, 9 percent (5 to 12). In red cells there was less

myristic acid than in serum, comparable amounts of palmitic acid, and twice as much palmitoleic and stearic acids. The unidentified acid with a retention time between 16:1 and 18:0 averaged about three percent of the total fatty acids in red cells, whereas serum triglycerides contained only trace amounts. The proportions of oleic and linoleic acids averaged about one-third to one-half less than values found for them in serum. In contrast to serum triglycerides which contained no fatty acids with chain lengths greater than 18 carbons or more polyunsaturated than linoleic acid, in red cells there were small amounts of linolenic, arachidic, and arachidonic acids. Unidentified longer chain acids with retention times greater than 20:4 also were present and equalled about five percent of the total fatty acids. The data for red cell triglycerides of these subjects showed good agreement with data reported by Hanahan et al. (42) (Table 4). The fatty acid patterns in triglycerides in white cells and platelets were quite similar to those in red cells.

In phospholipids, palmitic acid together with stearic, oleic, and arachidonic acids were present in highest concentrations in all fractions. Linoleic acid was high in serum and moderately high in red cells with only low amounts in white cells and platelets. The proportions of fatty acids in serum phospholipids of sixteen subjects (Tables 15 and 16) were quite comparable to the values reported by others (Table 2). The mean values (and ranges) of the major fatty

acids were: palmitic, 31 percent (27 to 36); stearic, 16 percent (12 to 19); oleic, 12 percent (10 to 16); linoleic, 24 percent (18 to 29); and arachidonic, 10 percent (7 to 14). The pattern of fatty acids found for phospholipids in red cells agreed with data reported by others (Table 3), but differed from that found in serum in the relative amounts of oleic and linoleic acids. In red cells, there was about one-third more oleic acid than in serum and one-half the amount of linoleic acid. As was true of cholesterol esters and triglycerides, the fatty acids of phospholipids in white cells and platelets were generally alike. In some respects, the fatty acid pattern in these cells resembled that in serum, whereas proportions of other fatty acids were more like those found in phospholipids of red cells. The concentrations of palmitic acid were slightly lower in white cells and platelets than in serum, and palmitoleic acid and the unidentified acid with a retention time between 16:1 and 18:0 averaged about four percent of the total fatty acids. In these cells, stearic and oleic acid values were comparable to those in serum, whereas there was only about one-fifth as much linoleic acid. There was more arachidic acid in white cells and platelets than in serum but similar amounts of arachidonic acid. Unidentified longer chain acids with retention times greater than 20:4 averaged about four percent of the total fatty acids in white cells and platelets.

The fatty acid composition of cholesterol esters, triglycerides,

and phospholipids of the four blood fractions for the one subject, ES, are presented in Tables 17, 18, and 19, respectively, and the means and standard errors of the means, in Table 20. In general, day-to-day variation of values for the one subject were equal to or greater than the individual-to-individual variations found among the sixteen subjects, as evaluated by a comparison of the standard errors of the means. Differences in the degrees of variation of specific fatty acids in the three lipid classes of each of the four fractions are more apparent in a graphic presentation of data for the major fatty acids from Tables 17, 18, and 19, Figures 1-3. Of all of the fatty acids plotted, palmitic (16:0) and stearic (18:0) varied the least. This was true for all lipid classes in all fractions. Oleic acid (18:1) showed the most variation. This was true not only in the cholesterol esters in all cells but also in triglycerides in red cells; the acid varied little in phospholipids. Concentrations of linoleic acid (18:2) varied slightly in cholesterol esters of serum and platelets and in phospholipids of serum but not in cholesterol esters of white cells, in triglycerides of all fractions, or in phospholipids in cells. Arachidonic acid (20:4), which was plotted only for phospholipids, varied in all fractions but to the greatest extent in red cells. Concentrations of several other acids, which were not plotted, also varied over quite wide ranges in certain classes: myristic acid (14:0) in triglycerides of red cells, palmitoleic acid (16:1) in triglycerides

Table 17. Percentages of Fatty Acids of Cholesterol Esters in the Blood Fractions of One Subject on Nine Different Days

Blood Fraction	Day	Fatty Acids												
		12:0	14:0	16:0	16:1	?	18:0	18:1	18:2	18:3	20:0	20:3	20:4	>20:4
Serum	1	tr		16	5	tr	3	19	55					
	2	tr	tr	16	3	2	3	19	51				5	
	3	tr	tr	15	4	tr	2	20	53	1			4	
	4	tr	tr	16	4	3	2	19	48				5	
	5	tr	tr	17	4	1	2	19	46				6	
	10	tr	tr	17	4	5	3	20	44			1	4	
	18	1	tr	20	5	3	2	21	43				5	
	25	tr	1	16	5	4	2	24	41			2	5	
	31		2	19	3	4	7	18	35	1			7	
White Cell	1	1	tr	30	8	9	13	19	4	tr	tr	2	tr	3
	2	1	tr	32	4	3	14	13	2	4	4			16
	3	2	2	27	9	4	12	19	5	1	1			4
	4	1	tr	22	9	9	7	8	3			1		35
	5	1	tr	32	10	8	13	16	2	1	1			5
	10	1	1	34	10	4	14	17	5	tr	tr			5
	18	tr	2	30	3	6	12	20	5					10
	25	tr	1	22	9	12	7	8	4	2	2		6	16
	31	1	1	20	8	14	7	14	5	1	1		4	17
Platelet	1	2	1	30	8	4	13	17	4	tr	tr	tr		4
	2	1	tr	20	3	4	9	32	7	tr	tr	3	3	8
	3[1]													
	4	1	tr	30	16	6	9	13	6	tr	tr	2		4
	5	2	2	31	10	9	8	11	4	2	2	2		8
	10	1	2	26	7	10	12	15	7		1	tr		11
	18	1	tr	24	12	5	14	21	5		tr		tr	8
	25	1	tr	27	8	6	12	23	5		1			8
	31	1	tr	22	6	tr	12	22	12			tr	tr	22

[1] No platelet sample.

Table 18. Percentages of Fatty Acids of Triglycerides in the Blood Fractions of One Subject on Nine Different Days

Blood Fraction	Day	Fatty Acids												
		12:0	14:0	16:0	16:1	?	18:0	18:1	18:2	18:3	20:0	20:3	20:4	>20:4
Serum	1	tr	19	32	5		6	33	5					
	2	tr	8	31	5		6	41	9					
	3	1	14	31	4		4	40	6					
	4	tr	11	31	5		5	37	11					
	5	tr	3	30	4	1	8	45	9					
	10	tr	11	28	6		6	40	8					
	18		2	27	4		8	50	10					
	25	tr	2	26	4		8	50	10					
	31		3	24	4		8	52	9					
Red Cell	1	2	2	27	15	2	13	25	4					
	2[1]													
	3	1	1	36	11	2	8	21	6	1	tr			8
	4	1	1	32	9	1	7	37	4	5	tr			
	5	2	2	24	8	4	11	18	7	3		2	2	5
	10	1	3	22	10	2	11	23	7	1	tr			13
	18	1	2	29	11	3	10	18	7	2	1	2		5
	25	2	tr	21	10	3	13	26	6	1	1	1		8
	31	1	2	15	5	1	6	57	3					7
White Cell	1	2	tr	26	6	4	11	11	2	5	5		2	14
	2	2	2	27	4	3	17	15	2	4	4	1	tr	8
	3	1	2	30	7	1	17	20	3	tr	2	tr		9
	4	3	1	27	12	2	12	15	3	tr	2	1	3	8
	5	2	2	27	4	2	17	19	3	tr	1			8
	10	2	1	30	14	3	12	22	3	tr	1	1	tr	5
	18	2	2	30	8	5	15	17	3	tr	1	1	tr	9
	25	1	tr	26	5	3	14	14	3	tr	2	tr	tr	11
	31	3	3	25	10	3	12	19	4	tr	3	2	tr	5
Platelet	1	1	1	32	9	3	12	15	4	2	2			4
	2	5	1	42	2	4	20	4		tr	tr	1	1	7
	3[2]													
	4	4	tr	36	9	4	12	14	2	tr	2	2		6
	5	1	1	34	13	2	15	16	2					8
	10	2	1	29	6	2	17	20	3	tr	2	tr		6
	18	2	1	32	7	3	14	14	4	1	2			10
	25	2	2	28	8	2	13	14	3	tr	2	2		11
	31	1	2	25	11	5	10	17	3	2	2			10

[1] No red cell sample.

[2] No platelet sample.

Table 19. Percentages of Fatty Acids of Phospholipids in the Blood Fractions of One Subject on Nine Different Days

Blood Fraction	Day	Fatty Acids												
		12:0	14:0	16:0	16:1	?	18:0	18:1	18:2	18:3	20:0	20:3	20:4	>20:4
Serum	1			29	2		17	13	26			5	8	
	2		tr	31	1		17	13	24	2			12	
	3		1	32	2		16	13	23			5	9	
	4			32	tr	tr	15	14	25				10	
	5		tr	37			18	13	25				6	
	10	tr	tr	31	1	2	16	13	18	tr	tr	5	12	
	18			28	2		16	13	25			1	13	
	25		tr	27	1		14	12	19		15	1	11	
	31		tr	30	1		17	13	25		1	2	12	
Red Cell	1		1	32	1	2	18	18	11				13	
	2		1	36	tr	tr	21	22	12				9	
	3		1	36			19	22	15		tr		7	
	4		1	34	tr	1	17	19	10		3	1	7	4
	5	tr	1	26	1	1	18	18	12			2	18	
	10		1	29	1		19	21	13			1	16	
	18			36	tr		21	25	13			tr	7	
	25	tr	1	28	1	3	17	20	12				17	
	31	tr	tr	29	1	2	14	19	12			2	15	3
White Cell	1	2	1	27	6	5	18	17	4	tr	3	4	7	
	2	3	2	25	5	tr	14	11	8	tr	14		14	
	3	4	2	23	4	6	16	9	2		13		9	
	4	2	tr	25	3	5	16	10	5		7	2	13	5
	5	2	1	29	2	1	19	9	4		5		12	8
	10	1	1	28	6	5	15	16	4		4		9	4
	18	2	1	26	3	4	17	14	6	tr	4	1	10	5
	25	7	tr	24	4	tr	16	10	tr		12			14
	31	3	tr	20	4	5	15	11	5		7	tr	12	9
Platelet	1	2	2	29	4	tr	16	15	2		4	4	8	
	2	2		27	4	3	20	13	5		5	tr	7	5
	3[1]													
	4	4	1	21	2	5	17	9	3	tr	7	tr	10	9
	5	3	1	26	2	5	17	10	3		6	tr	11	8
	10	6	6	25	2	4	12	18	4	2	2		8	8
	18	5	1	26	3	5	16	10	4		8	tr	8	8
	25	3	1	22	4	3	15	12	4		7	tr	11	10
	31	4	1	23	4	6	15	12	4	tr	6	3	14	tr

[1] No platelet sample.

Table 20. The Mean Percentages of Fatty Acids of Three Lipid Classes in the Blood Fractions of One Subject on Nine Different Days

Lipid Class	Fatty Acids												
	12:0	14:0	16:0	16:1	?	18:0	18:1	18:2	18:3	20:0	20:3	20:4	>20:4
Cholesterol Esters													
Serum	0-1	0-2	17±0.6[1]	4±0.3	2±0.6	3±0.6	20±0.6	46±2.1	0-1		0-2	5±0.4	
Red Cell[2]													
White Cell	tr-2	tr-2	28±1.7	8±0.9	8±1.2	11±1.1	15±1.5	4±0.5	0-4	0-4	0-2	0-6	12±3.4
Platelet	1-2	tr-2	26±1.4	9±1.4	6±1.1	11±0.8	19±2.4	6±0.9	0-2	0-2	0-3	0-3	9±2.0
Triglycerides													
Serum	0-1	8±2.0	29±0.9	5±0.3	0-1	7±0.5	43±2.2	9±0.7					
Red Cell	1-2	2±0.4	26±2.4	10±1.0	2±0.4	10±0.9	28±4.7	6±0.6	2±0.7	0-1	0-2	0-2	8±1.3
White Cell	1-3	1±0.3	28±0.7	8±1.2	3±0.3	14±0.8	17±1.2	3±0.3	tr-5	2±0.5	0-2	0-3	9±0.9
Platelet	1-5	1±0.4	32±1.9	8±1.2	3±0.4	14±1.1	14±1.6	3±0.4	0-2	0-2	0-2	0-1	8±0.9
Phospholipids													
Serum	0-1	0-1	31±1.0	1±0.4	0-2	16±0.5	13±0.3	23±0.9	0-2	0-15	0-5	10±0.7	
Red Cell	0-1	0-1	32±1.3	0-1	0-3	18±0.7	20±0.7	12±0.5		0-3	0-2	12±1.5	0-4
White Cell	1-7	tr-2	25±0.9	4±0.5	3±0.8	16±0.6	12±1.0	4±0.7	0-1	8±1.4	0-4	11±0.9	8±1.5
Platelet	2-6	0-6	25±0.9	3±0.4	4±0.7	16±0.8	12±1.1	4±0.4	0-2	6±0.7	0-4	10±0.8	7±1.3

[1] Standard error of the mean (87).

[2] No cholesterol ester sample.

Figures 1 - 3.

1. Major Fatty Acids in Cholesterol Esters of the Blood Fractions of One Subject on Nine Different Days
2. Major Fatty Acids in Triglycerides of the Blood Fractions of One Subject on Nine Different Days
3. Major Fatty Acids in Phospholipids of the Blood Fractions of One Subject on Nine Different Days

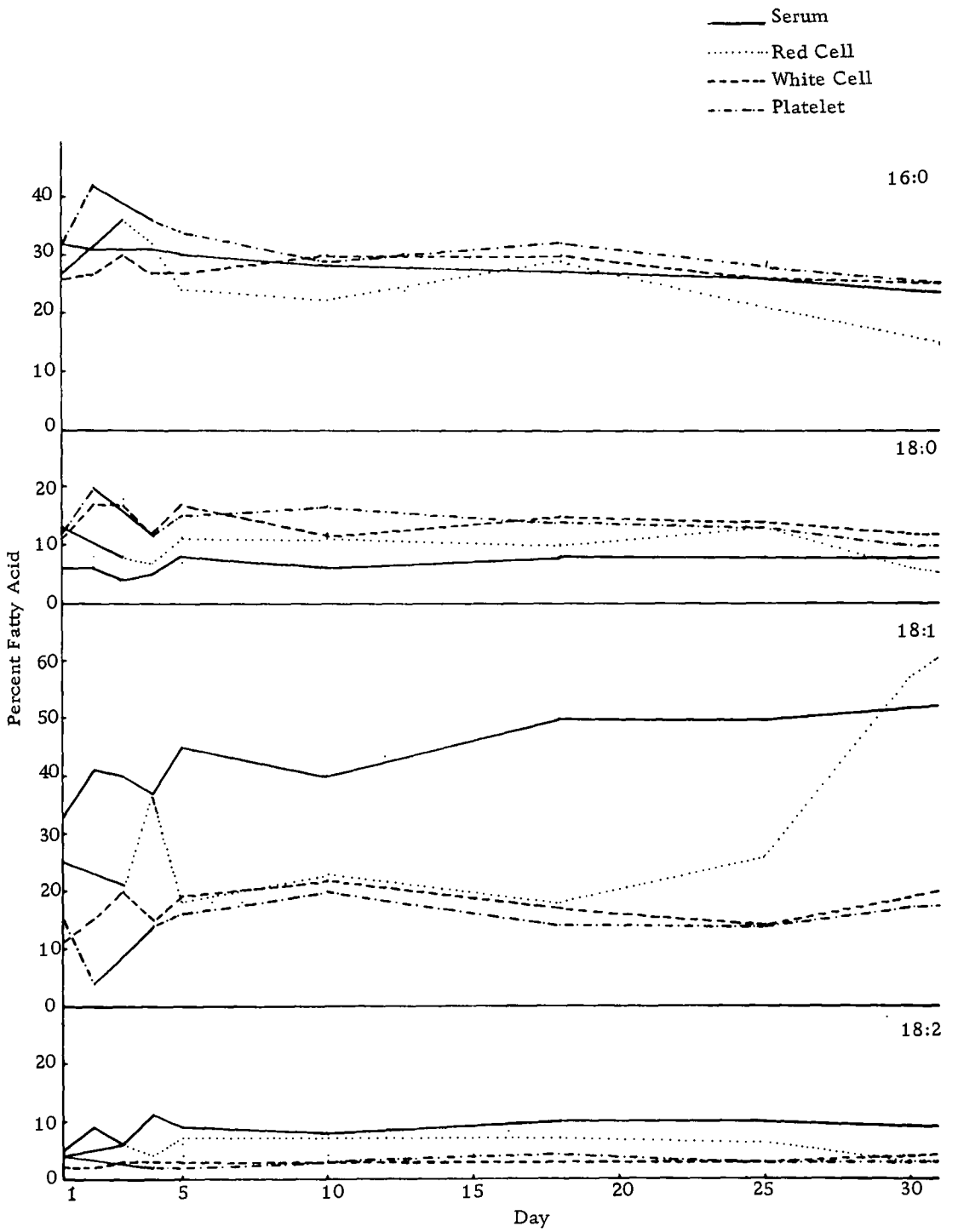
Serum
Red Cell
White Cell
Platelet
16:0
18:0
18:1
18:2
Percent Fatty Acid
Day
0
10
20
30
40
50
60
1
5
10
15
20
25
30

Figure 2.

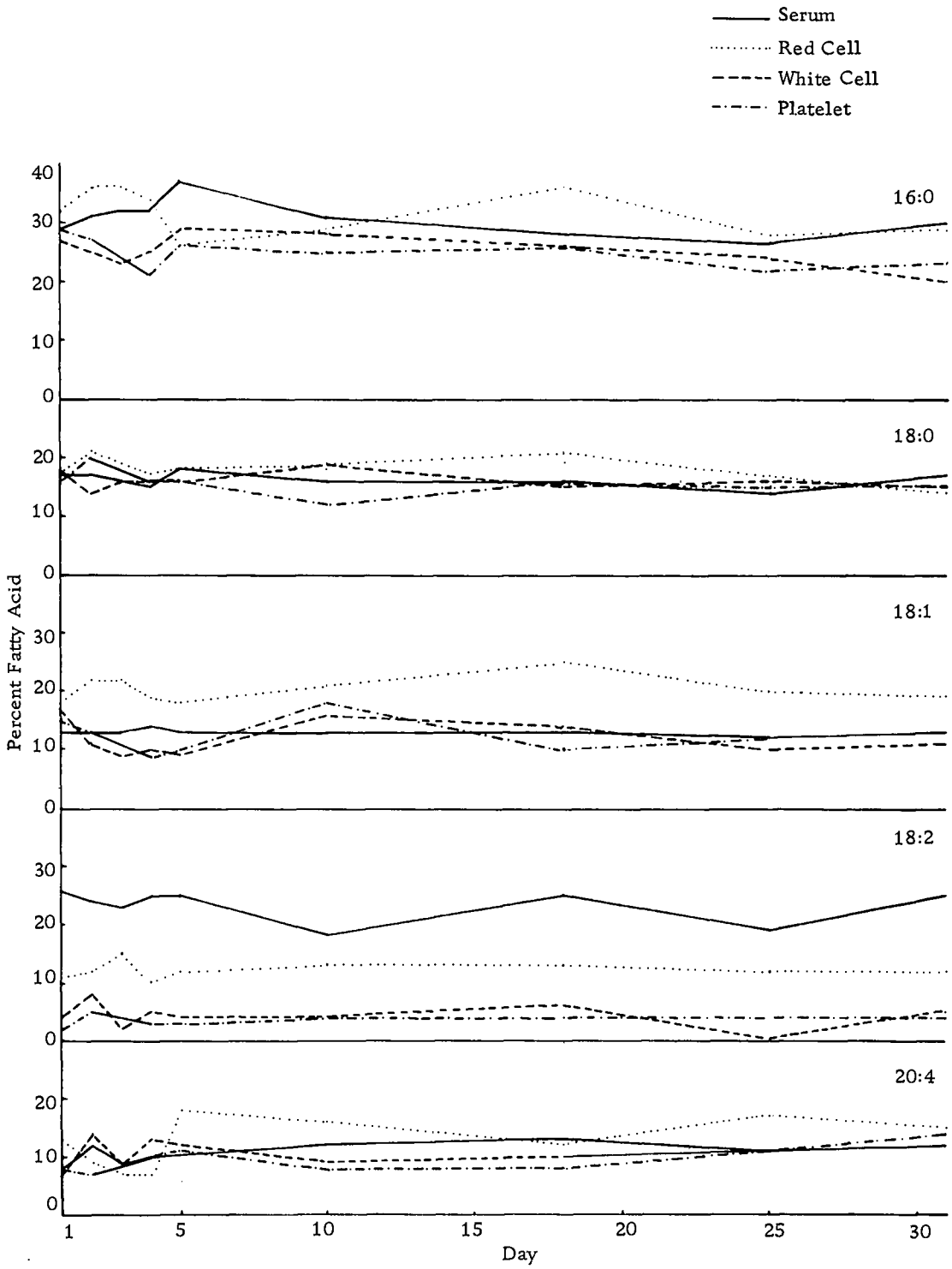
Serum
Red Cell
White Cell
Platelet
16:0
18:0
18:1
18:2
20:4
Percent Fatty Acid
Day
1
5
10
15
20
25
30
0
10
20
30
40

Figure 3.

of cell fractions, and the unidentified longer chain acids with retention times greater than 20:4 in all lipid classes in all cells (Table 20).

For the sixteen subjects the mean values for only three acids showed greater variability than the mean values for the one subject: myristic acid in cholesterol esters in serum, arachidonic acid in phospholipids of cells, and the unidentified longer chain acids in phospholipids of cells (Table 16). There were, in addition, a few samples of lipid classes which contained distinctly higher amounts of certain fatty acids. For example, the unidentified acid with a retention time between 16:1 and 18:0 in cholesterol esters of white cells for subject SP was 19 percent, palmitoleic acid in cholesterol esters of platelets for subject MB was 20 percent, and linolenic acid of phospholipids in red cells for subject MF was 12 percent (Tables 13 and 15).

Several investigators have reported that, in animals, marked changes in the dietary intake of fat affected the fatty acid composition of lipid classes, particularly phospholipids (68, 69, 99). In this study there was no apparent relationship between fatty acid composition of any lipid class and fat intake. However, among these subjects the fat intake was quite similar in both quantity and quality.

It has been reported that the fatty acid composition of cholesterol esters of many different human organs is strikingly similar

and that the patterns of triglycerides and phospholipids are also rather characteristic for each of these classes (96). However, the data obtained in this study on the composition of fatty acids in the major lipid classes of the four blood fractions of normal subjects did not support this belief.

The fatty acids of the different lipid classes in blood fractions were compared with data which have been reported for human tissues, e.g., liver, spleen, heart, kidney, aorta intima, testis, perinephric fat, adipose tissue, and atherosclerotic plaques (44, 84, 96). It was found that the fatty acid patterns in triglycerides of all blood fractions and of several tissues were similar. The serum fraction of blood most accurately reflected the fatty acid pattern of triglycerides of other tissues, since in cells there was somewhat more stearic acid and somewhat less oleic acid. In addition, in blood cells, the unidentified longer chain acids with retention times greater than 20:4 were present, which were essentially absent from other tissues. In phospholipids, the patterns of fatty acids of blood cells were more like those of other tissues; phospholipids in serum contained a higher linoleic acid concentration. In cholesterol esters, the proportions of fatty acids differed not only among the blood fractions but also among certain other tissues. If blood analysis is used as one means of assessing the kinds and the relative amounts of fatty acids combined with lipid classes of other tissues, it would appear that more

inclusive data would be obtained by analyses of all of the blood fractions rather than only of serum, which is the usual practice.

SUMMARY AND CONCLUSIONS

The concentrations of total lipid, the distributions of lipid classes, and the fatty acid compositions of cholesterol esters, triglycerides, and phospholipids were determined in serum, red cells, white cells, and platelets isolated from venous blood of sixteen adult men and women. In addition, nine samples of blood obtained from one subject over a four-week period were analyzed for these same lipids.

The formed elements were isolated as three separate fractions by the modification of a procedure which permitted the simultaneous separation of red cells, white cells, and platelets from the same sample of blood. Serum was separated in the usual manner. Satisfactory procedures for lipid extraction were then developed for each blood fraction by modifications of published methods. The proportional distribution of lipid classes in each fraction was quantitated. Preparative thin-layer chromatography was used to separate the total lipid mixtures into major lipid classes and methyl esters of fatty acids of cholesterol esters, triglycerides, and phospholipids were prepared by direct interesterification and determined by gas-liquid chromatography.

The amounts of total lipid in sera and in red cells were similar, averaging 603 and 555 mg per 100 ml, respectively. White

cells and platelets contained two to four times as much, averaging 3051 and 2587 mg per 100 gm, respectively. The concentrations of total lipid in sera and red cells were essentially comparable to values reported in the literature as normal, whereas the amounts of total lipid determined in white cells were higher than values previously reported. Total lipids in platelets were equal to or slightly less than values in white cells. From the concentration of lipid in one blood fraction, it was not possible to predict whether lipids in other fractions would be high or low.

Certain characteristic distributions of lipid classes were found for the different blood fractions. Serum was characterized by high cholesterol ester and triglyceride concentrations, together with phospholipids. In red cells, phospholipids and free cholesterol comprised most of the total lipid, whereas there were generally lower amounts of triglycerides than in serum, and almost no cholesterol esters. Also in red cells, there was a relatively high concentration of an unidentified component, presumably a hydrocarbon-like compound. Lipid classes in white cells and platelets were similar to red cells in containing high amounts of phospholipids and free cholesterol and some of the unidentified component. In contrast to red cells, in white cells and platelets there were higher amounts of cholesterol esters and free fatty acids.

The patterns of fatty acids which comprise the lipid classes

were not constant from fraction to fraction. The proportions of fatty acids in triglycerides were more similar among all fractions than were the proportions of fatty acids in cholesterol esters or phospholipids. Palmitic and oleic acids were the major fatty acids in triglycerides. In phospholipids, palmitic acid together with stearic, oleic, and arachidonic acids were present in high concentrations in all blood fractions. The concentrations of linoleic acid, however, varied. It was high in serum, moderately high in red cells, and low in white cells and platelets. The greatest variations in proportions of fatty acids among the blood fractions occurred in cholesterol esters. Most noteworthy were the low amounts of linoleic acid in white cells and platelets compared to the high amount in serum. Cellular fractions, in general, were characterized by containing significant amounts of unidentified longer chain acids with retention times greater than 20:4 in all lipid classes. These were absent in all classes of serum.

Individual-to-individual variations were greater than day-to-day variations for values of total lipids in serum and red cells, of all lipid classes in serum, and of phospholipids in all fractions. However, the total lipids of white cells and platelets, the concentrations of cholesterol esters, free cholesterol, and triglycerides in cells, and the percentages of fatty acids in the lipid classes of all fractions of the one subject showed variations that were equal to or

greater than individual variations among the sixteen subjects.

There was no consistent relationship between the concentrations of any lipids and the age or sex of the subjects. Similarly, there was no relationship found between the lipid values and fat intake as evaluated by the diet questionnaire and the one-day diet record, either among the sixteen subjects or day to day for the one subject.

Differences in the distribution of lipid classes and the fatty acid composition of lipid classes among the four blood fractions for these healthy subjects suggest the desirability of concurrent analyses of all blood fractions in studies of lipid metabolism in humans. The significance of the person-to-person variation in the proportions of fatty acids in phospholipids might be clarified by fractionating the total phospholipid class into its individual classes and determining the fatty acids combined in each. It would be expected that the value of analyses of the several blood fractions could be better evaluated if data on lipid concentrations of all fractions of blood also could be obtained from subjects with known disorders of lipid metabolism.

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APPENDICES

APPENDIX I.

A. Red and White Cell Counts and Differential Counts for Sixteen Subjects

B. Red and White Cell Counts and Differential Counts for One Subject on Nine Different Days

A. Red and White Cell Counts and Differential Counts for Sixteen Subjects

			Leucocytes					
Subject	Red Cells per cmm x 10^6	White Cells per cmm x 10^3	Polymorphonuclear Neutrophils %	Bands %	Lymphocytes %	Monocytes %	Eosinophils %	Basophils %
KG	5.0	6.9	54	1	44	1	3	0
MM	4.8	8.8	47	0	47	2	5	0
SP	4.4	6.6	52	1	33	4	9	1
ES	5.6	8.3	65	0	31	0	4	0
DB	5.1	7.8	69	0	27	1	3	0
BH	5.7	8.0	55	2	35	6	2	0
EJ	5.5	7.8	67	0	30	1	2	0
MB	5.1	7.2	47	0	41	1	11	0
MF	5.2	8.5	64	0	26	4	6	0
CG	5.3	7.5	47	1	49	4	1	0
RM	5.6	9.1	59	0	32	4	6	0
KP	5.8	7.6	51	1	36	4	8	1
JJ	5.8	8.4	56	1	33	4	7	0
SB	6.1	8.4	57	0	34	6	3	0
HH	5.1	10.6	64	0	30	3	3	1
EB[1]								

[1] No red cell and white counts and differential counts.

B. Red and White Cell Counts and Differential Counts for One Subject on Nine Different Days

Day	Red Cells per cmm x 10^6	White Cells per cmm x 10^3	Leucocytes: Polymorphonuclear Neutrophils %	Bands %	Lymphocytes %	Monocytes %	Eosinophils %	Basophils %
1	5.6	8.3	65	0	31	0	4	0
2	5.5	6.8	57	0	38	3	2	0
3	5.2	7.0	53	0	44	1	2	0
4	5.5	6.9	55	0	37	4	4	0
5	5.5	7.2	57	0	35	5	3	0
10	5.0	7.2	58	0	35	4	3	0
18	5.1	6.9	51	0	43	2	4	0
25[1]	5.1	7.1						
31	5.3	7.5	56	0	38	1	5	0

[1] Blood smears for differential counts not counted.

APPENDIX II.

Diet Questionnaire

DIET QUESTIONNAIRE

1. How often do you eat the following foods?

	Never or Practically Never	No. of Servings	Per: Day	Per: Week	Per: Month
A. Leafy or green vegetables:					
Lettuce	____	____	____	____	____
Spinach	____	____	____	____	____
Broccoli	____	____	____	____	____
Asparagus	____	____	____	____	____
Kale	____	____	____	____	____
Turnip Greens	____	____	____	____	____
Beet Greens	____	____	____	____	____
Mustard Greens	____	____	____	____	____
Other_____	____	____	____	____	____
Yellow Vegetables:					
Carrots	____	____	____	____	____
Squash	____	____	____	____	____
Sweet Potatoes	____	____	____	____	____
Pumpkin	____	____	____	____	____
Cantaloup	____	____	____	____	____
Other_____	____	____	____	____	____
B. Citrus fruit or raw vegetables:					
Oranges	____	____	____	____	____
Grapefruit	____	____	____	____	____
Tomatoes	____	____	____	____	____
Raw Cabbage	____	____	____	____	____
Other_____	____	____	____	____	____
C. Potatoes (white):					
Baked	____	____	____	____	____
Mashed	____	____	____	____	____
Boiled	____	____	____	____	____
Fried	____	____	____	____	____
Other_____	____	____	____	____	____
D. Other fruits and vegetables:					
Apples	____	____	____	____	____
Peaches	____	____	____	____	____
Pineapple	____	____	____	____	____
Prunes	____	____	____	____	____

	Never or Practically Never	No. of Servings	Per: Day	Per: Week	Per: Month
Pears	____	____	____	____	____
Plums	____	____	____	____	____
Other_____	____	____	____	____	____
Green Beans	____	____	____	____	____
Corn	____	____	____	____	____
Peas	____	____	____	____	____
Cauliflower	____	____	____	____	____
Beets	____	____	____	____	____
Other_____	____	____	____	____	____
E. Meat:					
Bacon	____	____	____	____	____
Ham	____	____	____	____	____
Veal	____	____	____	____	____
Beef	____	____	____	____	____
Lamb or Mutton	____	____	____	____	____
Pork	____	____	____	____	____
Organ Meats (e.g. liver)	____	____	____	____	____
Deer or Elk	____	____	____	____	____
Other_____	____	____	____	____	____
Poultry:					
Chicken	____	____	____	____	____
Turkey	____	____	____	____	____
Other_____	____	____	____	____	____
Fish and Sea Foods:					
Fresh water fish (e.g. trout)	____	____	____	____	____
Sea fish (e.g. salmon, sole, halibut, snapper)	____	____	____	____	____
Canned Tunafish	____	____	____	____	____
Shrimp	____	____	____	____	____
Clams	____	____	____	____	____
Oysters	____	____	____	____	____
Other_____	____	____	____	____	____
F. Eggs:					
Scrambled	____	____	____	____	____
Fried	____	____	____	____	____
Boiled	____	____	____	____	____

	Never or Practically Never	No. of Servings	Per: Day	Per: Week	Per: Month
Omelet	____	____	____	____	____
Other_____	____	____	____	____	____
G. Legumes:					
Dried Peas	____	____	____	____	____
Dried Beans	____	____	____	____	____
H. Bread or Toast:					
White	____	____	____	____	____
Brown	____	____	____	____	____
Other_____	____	____	____	____	____
I. Cereals:					
Prepared:					
Cornflakes	____	____	____	____	____
Wheaties	____	____	____	____	____
Oat	____	____	____	____	____
Rice	____	____	____	____	____
Bran	____	____	____	____	____
Bran Flakes	____	____	____	____	____
Other_____	____	____	____	____	____
Cooked:					
Oatmeal	____	____	____	____	____
Wheat	____	____	____	____	____
Rice	____	____	____	____	____
Other_____	____	____	____	____	____
J. Flour Products:					
Biscuits	____	____	____	____	____
Pastries	____	____	____	____	____
Muffins	____	____	____	____	____
Pancakes	____	____	____	____	____
Waffles	____	____	____	____	____
Spaghetti	____	____	____	____	____
Macaroni	____	____	____	____	____
Pizza	____	____	____	____	____
Other_____	____	____	____	____	____
K. Butter	____	____Tbsp		____	____
Margarine	____	____Tbsp		____	____
Brand:_____					
_____					

	Never or Practically Never	No. of Servings		Per: Day	Week	Month
L. Milk:						
Whole (4%)	____	____	Cups	____	____	____
Low Fat (2%)	____	____	Cups	____	____	____
Skim	____	____	Cups	____	____	____
Skim (Dried)	____	____	Cups	____	____	____
Other_____	____	____	Cups	____	____	____
Cheese:						
Cheddar, etc.	____	____		____	____	____
Cottage Cheese	____	____		____	____	____
Other_____	____	____		____	____	____
Ice Cream:						
Ice Milk	____	____		____	____	____
Low Fat Ice Cream	____	____		____	____	____
Other_____	____	____		____	____	____
M. Cream:						
Coffee Cream	____	____	Tbsp	____	____	____
Whipping Cream	____	____	Tbsp	____	____	____
Sour Cream	____	____	Tbsp	____	____	____
Other_____	____	____	Tbsp	____	____	____
N. Miscellaneous:						
Jams	____	____	Tbsp	____	____	____
Jellies	____	____	Tbsp	____	____	____
Honey	____	____	Tbsp	____	____	____
Syrup	____	____	Tbsp	____	____	____
Peanut Butter	____	____	Tbsp	____	____	____
Nuts	____	____	1/4C.	____	____	____

2. Are your meats and other foods generally: Baked____ Fried____ Broiled____ ?

3. Do you frequently eat gravies and sauces made with fat?
____(Yes) ____(No) ____(Never)

If yes: Kind_____ Servings____Tbsp Per Day____ Week____ Month____
_____ ____Tbsp ____ ____ ____
_____ ____Tbsp ____ ____ ____

4. What kind of salad dressings do you use?

Home Made_____	____	____	Tbsp	____	____	____
_____	____	____	Tbsp	____	____	____
Prepared_____	____	____	Tbsp	____	____	____
_____	____	____	Tbsp	____	____	____

	Never or Practically Never	No. of Servings	Per: Day	Week	Month
5. Do you generally eat desserts? _____(Yes) _____(No)					
Pies	____	____	____	____	____
Cakes	____	____	____	____	____
Cookies	____	____	____	____	____
Puddings	____	____	____	____	____
Fruit (Raw)	____	____	____	____	____
Fruit (Cooked or Canned)	____	____	____	____	____
Jello	____	____	____	____	____
Other_____	____	____	____	____	____
6. Do you generally eat between meals (snacks)? ____(Yes) ____(No)					
Fruit	____	____	____	____	____
Juice	____	____	____	____	____
Milk	____	____	____	____	____
Milkshakes	____	____	____	____	____
Nuts	____	____	____	____	____
Potato Chips	____	____	____	____	____
Candy	____	____	____	____	____
Soft Drinks	____	____	____	____	____
Popcorn	____	____	____	____	____
Other_____	____	____	____	____	____
_____	____	____	____	____	____
7. Which beverages do you generally drink? (Coffee, tea, milk, cocoa, soft drinks, alcoholic)					
Breakfast_____	____	____	____	____	____
_____	____	____	____	____	____
Lunch_____	____	____	____	____	____
_____	____	____	____	____	____
Dinner_____	____	____	____	____	____
_____	____	____	____	____	____
Other_____	____	____	____	____	____
_____	____	____	____	____	____

8. How much sugar do you consume each day? _____Tbsp
(In coffee, on cereals, on fruits or other uses)

9. What kind of fats do you use?

Lard_____	Crisco_____
Butter_____	Spry_____
Cream_____	Wesson Oil_____
Bacon Drippings_____	Mazola_____
	Others_____

(Female)

10. Has there been any significant or marked change in the kinds of fats eaten from childhood until now?_____

_____

_____

11. Have you consciously made any shift from your usual use of fats to the more polyunsaturated fats in the past few years?_____
If yes, how?_____

_____

If yes, why?____Advertising; ____Medical; ____Cooking Quality; ____Other

12. Are there any foods which are limited in your diet?_____
If yes, which foods?_____

13. Do you have any known organic disorder at this time?_____
If yes, what?_____

14. Are you currently receiving therapeutic treatment?_____
If yes, nature of treatment_____

15. Do you take thyroid or any other medication daily?_____
If yes, specify_____

16. Have you passed the menopause?_____ If not, what was the approximate date of your last menstrual period?_____

_____

COMMENTS:

Name_____ Date_____ Age_____
Height_____
Weight_____

(Male)

10. Has there been any significant or marked change in the kinds of fats eaten from childhood until now?_____

_____

_____

11. Have you consciously made any shift from your usual use of fats to the more polyunsaturated fats in the past few years?_____
If yes, how?_____

_____

If yes, why?____Advertising; ____Medical; ____Cooking Quality; ____Other

12. Are there any foods which are limited in your diet?_____
If yes, which foods?_____

13. Do you have any known organic disorder at this time?_____
If yes, what?_____

14. Are you currently receiving therapeutic treatment?_____
If yes, nature of treatment_____

15. Do you take thyroid or any other medication daily?_____
If yes, specify_____

COMMENTS:

Name_____ Date_____ Age_____
Height_____
Weight_____

APPENDIX III.[1]

A. Analyses of the Diet Questionnaire and the One-Day Diet Record for Sixteen Subjects

B. Analyses of the One-Day Diet Records for One Subject on Nine Different Days

[1]Values calculated by G. Woosley.

A. Analyses of the Diet Questionnaire and the One-Day Diet Record of Sixteen Subjects

	Diet Questionnaire		One-Day Diet Record													
Subject	Diet Score	Estimate of Fat Intake	Diet Score	Total Calories	Protein gm	Carbo-hydrate gm	Fat gm	Total Sat. Fatty Acids gm	14:0 gm	16:0 gm	18:0 gm	Total Unsat. Fatty Acids gm	18:1 gm	18:2 gm	18:3 gm	Other gm
KG	95	Mod.	63	2654	84	263	144	57	5	33	16	79	67	7	1	4
MM	100	Mod-High	90	4049	137	426	209	90	14	48	24	108	90	10	1	6
SP	100	Mod-Low	85	2186	89	244	101	39	7	22	8	55	35	18	1	1
ES	100	Mod-Low	60	1361	67	158	50	22	5	11	4	18	18	4	tr	1
DB	95	Mod-Low	100	1728	76	225	62	24	4	14	5	31	22	9	tr	1
BH	85	Mod-Low	70	1598	94	147	72	22	4	12	5	42	25	17	1	1
EJ	98	Mod-Low	58	1944	76	207	96	41	3	22	14	46	39	8	tr	1
MB	78	Mod-Low	68	1538	75	119	86	27	1	16	9	53	32	15	tr	5
MF	98	Mod-Low	95	1987	72	255	79	35	6	18	9	38	30	4	tr	3
CG	83	Mod-High	100	4126	130	463	196	80	11	44	21	104	89	10	1	4
RM	100	Mod-High	90	4049	137	426	209	90	14	48	24	108	90	10	1	6
KP	100	Mod.	80	2561	129	233	128	48	7	28	10	72	48	20	1	3
JJ	88	Mod-High	98	3630	155	278	210	97	5	53	35	101	93	6	tr	2
SB	100	Mod.	85	3359	128	435	128	45	6	27	9	71	51	22	tr	1
HH	100	Mod-High	85	2703	82	317	134	60	9	33	15	66	44	20	1	2
EB	88	Mod.	70	2410	106	213	129	41	1	24	13	81	50	23	tr	8

B. Analyses of One-Day Diet Records for One Subject on Nine Different Days

	One-Day Diet Record													
Day	Diet Score	Total Calories	Protein gm	Carbo-hydrate gm	Fat gm	Total Sat. Fatty Acids gm	14:0 gm	16:0 gm	18:0 gm	Total Unsat. Fatty Acids gm	18:1 gm	18:2 gm	18:3 gm	Other gm
1	60	1361	67	158	50	22	5	11	4	18	18	4	tr	1
2	80	2085	77	226	102	31	1	22	8	63	42	20	tr	2
3	90	2660	88	292	130	41	1	28	11	83	56	26	tr	1
4	100	2811	94	302	148	55	5	33	16	86	64	16	tr	6
5	100	1836	83	190	80	32	2	19	9	43	35	5	tr	3
10	75	2133	67	319	69	25	3	14	7	40	35	4	tr	1
18	85	1946	75	213	91	32	3	19	8	55	41	10	tr	4
25	83	1730	56	183	81	30	4	17	7	46	35	7	tr	3
31	75	2086	75	265	85	32	2	19	10	47	39	7	tr	1