CLINICAL STUDIES OF BLOOD LIPID METABOLISM
I. NORMAL BLOOD LIPID VARIATIONS OF PHOSPHOLIPIDS, NEUTRAL FATS, TOTAL LIPIDS, AND LIPID FRACTION PERCENTAGES

A. ALLEN GOLDBLOOM, M. D., F. A. C. P. New York, N. Y.

MUCH HAS BEEN expressed of the effect of lipids upon conditions such as atherosclerosis, arteriosclerosis, coronary disease, and liver pathology. Experimental and clinical studies have been published with no final and with varied conclusions. The lay press has stressed these indefinite results to such an extent that patients request and demand to know "The values of their fats in the blood."

Without a doubt, changes in the arteries of animals have been produced experimentally simulating atherosclerotic processes in humans.

Blood studies, especially of cholesterol, are numerous. Other lipid substances such as phospholipids (phosphatides) and fatty acids are less frequent. Some are so persistent as to state, with a note of finality, that a ratio of these substances, for instance, phospholipids to cholesterol or vice versa, in one way or another speak for or against atherosclerosis or overt coronary disease. The more exact and most recent work is that of Gofman and his associates with their qualitative study of lipomicrons (any fat particle) and chylomicrons (large fat particles) by ultra centrifugation. This awaits further confirmation for practical clinic usage.

Before any discussion of the clinical value of lipids is mentioned, it seems in order to define lipids; the various types commonly encountered clinically; their function; and normal variations, at this stage of our knowledge. In subsequent publications, the author will attempt to evaluate findings in certain clinical conditions.

CLASSIFICATION OF LIPIDS (1)

The term "lipid" (2) is used to designate those substances which, in their general properties and particularly in their solubilities resemble fats.

1. *True fats and oils*, triglycerides of fatty acids.
2. *Nitrogen-containing lipids*, substances of a fat-like nature, yielding on hydrolysis fatty acids or derivatives of fatty acids and containing nitrogen.
   - A. *Phospholipids*, nitrogenous lipids containing phosphorus.
     - a. *Phosphatides*, triglycerides in which one fatty acid is replaced by a phosphoric acid tester. Each molecule contains two molecules of fatty acid to one of phosphoric acid. To this group belong lecithin and the cephalins.
     - b. *Sphingomyelins*, like the phosphatides contain both nitrogen and phosphorus, but only a single molecule of fatty acid to each molecule of phosphoric acid.
   - B. *Cerebrosides*, substances containing fatty acids, nitrogen, and a carbohydrate group, but no phosphorus. Of these, at least two members have been identified, phrenosin and kerasin.
3. *Unsaponifiable materials*, having no close chemical relation to fat except similar solubilities, and no functional relationship except that some members form esters with fatty acids.
   - A. *Sterols and Steroids*. Compounds having a perhydrocyclopentophenanthrene nucleus.
     - a. cholesterol and its immediate derivatives.
     - b. steroid vitamins and provitamins.
     - c. steroid hormones.
     - d. bile acids.

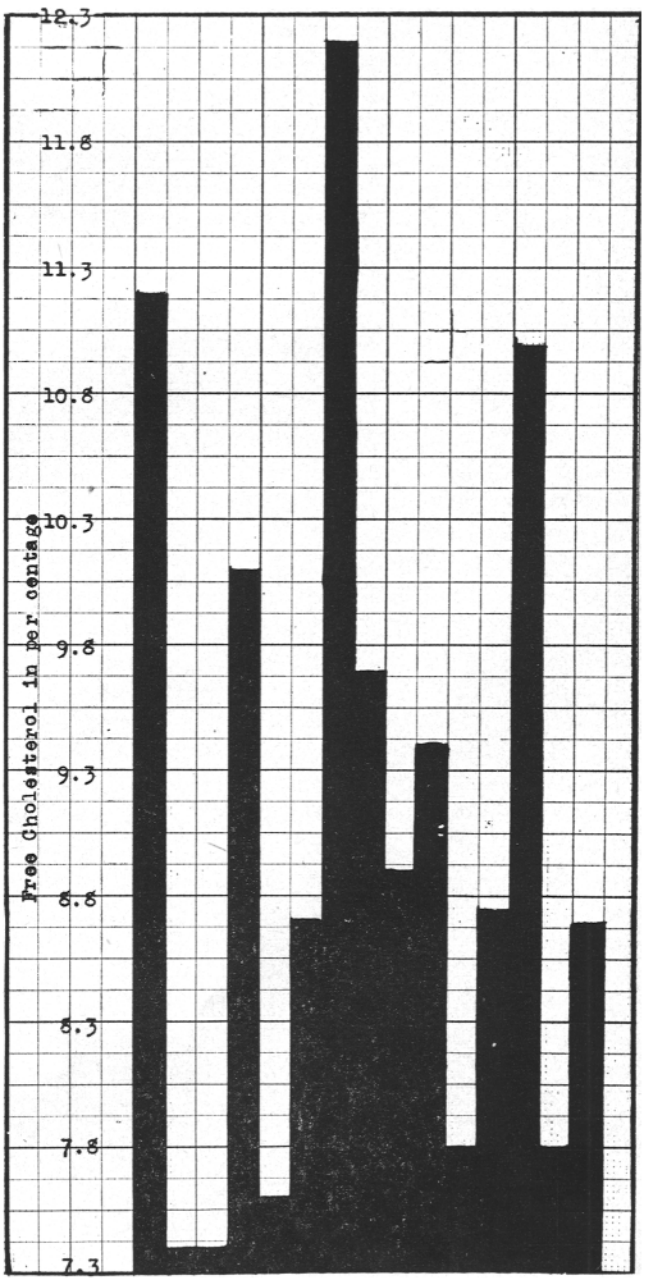

Fig. 1: Histogram showing the percentage of serum free cholesterol in terms of total lipid concentration in normal male and female patients.

From the Medical Services of the Beth Israel Hospital and the New York Medical College, Flower and Fifth Avenue Hospital (Metropolitan Hospital Division).

Submitted July 7, 1951.

B. *Other fat soluble vitamins and provitamins.*
   a. carotenes and A vitamins.
   b. tocopherols and E vitamins.
   c. naphthaquinones and K vitamins.

C. *Unidentified nonsaponifiable materials.*

GENERAL TERMINOLOGY

Ester—is any compound formed from an alcohol and an acid by the removal of water.

$CH_3COOH + C_2H_5OH = CH_3COOC_2H_5 + H_2O$

Acetic acid + alcohol = ethyl acetate (ester)

Glyceride—is an organic acid ester of glycerol (glycerin). The natural fats are glycerides of the higher fatty acids and usually are mixed glycerides.

$H_2C - O - CO - C_{17}H_{35}$
$H\ C - O - CO - C_{17}H_{35}$
$H_2C - O - CO - C_{17}H_{35}$

(Tristearin)

A Simple Glyceride

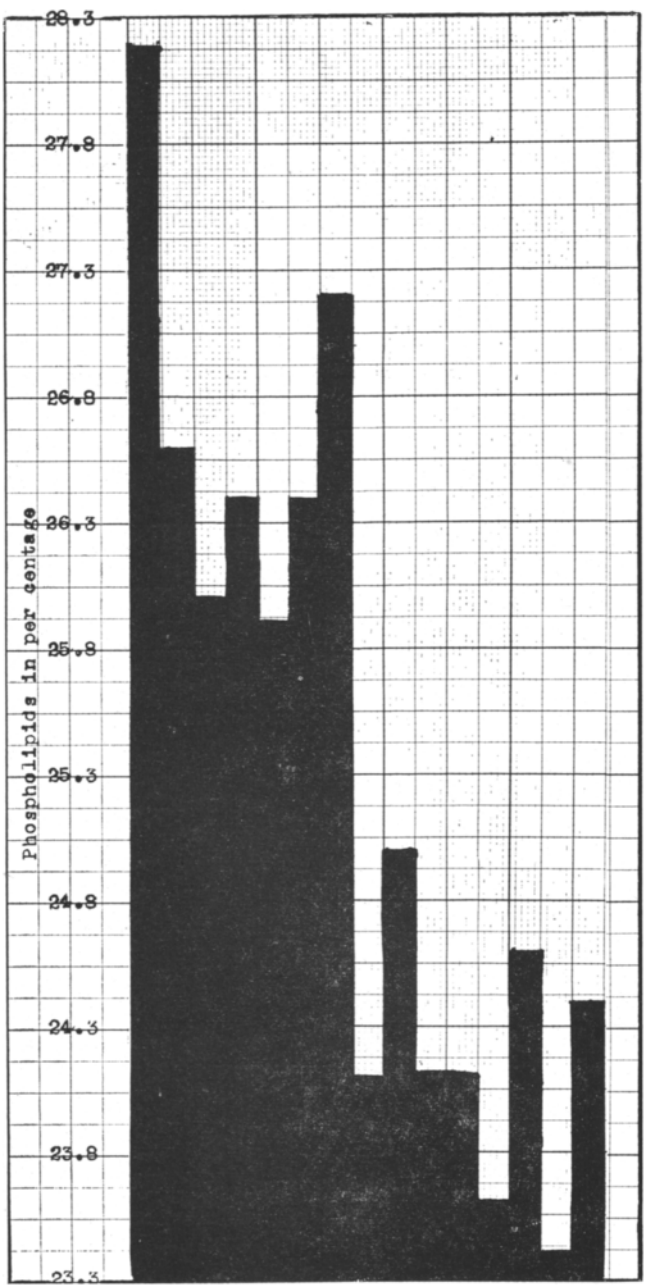

Fig. 2: Histogram showing percentage of phospholipids, as part of total lipid concentration, in normal patients.

Fats (3)—are esters of fatty acids and glycerols (glycerin) that contain mixtures of glycerides which are esters of glycerol and one or more fatty acids.

$H_2C - O - CO - R_1$
$H\ C - O - CO - R_2$
$H_2C - O - CO - R_3$

General formula for fat. R_1 etc. nomenclature for fatty acid radical.

Fatty Acids (3)—are monobasic aliphatic (pertaining to an oil) acid formed by the oxidation of a primary alcohol and having the formula $C_nH_{2n}O_2$. Example: $C_{15}H_{31}COOH$ (palmitic acid) or $(C_{16}H_{32}O_2)$.

Some of the fatty acids found in fats which occur more or less commonly are acetic (vinegar), butyric (butter), arachidic (peanut oil), etc. The most important and most widely distributed fatty acids are stearic, palmitic, and oleic. The fatty acids form soaps with metals or alkalies, as:

$C_3H_5(O\text{-}CO\text{-}C_{17}H_{35})_3 + 3NaOH = 3C_{17}H_{35}COONa + C_3H_5(OH)_3$

stearin + sodium hydroxide = sodium stearate (soap) + glycerol (glycerin) and esters with alcohol

$CH_3COOH + C_2H_5OH = CH_3COOC_2H_5 + H_2O$

acetic acid + alcohol = ethyl acetate (an ester).

Neutral fats (4)—are defined when all three alcoholic groups of glycerol are esterified with fatty acids (R_1, R_2 or R_3) which may be either saturated or unsaturated.

$H_2C - O - CO - C_{15}H_{31}$
$H\ C - O - CO - C_{15}H_{31}$
$H_2C - O - CO - C_{15}H_{31}$

Palmitin (neutral fat)

The general structure of fats is that of glycerol esters. They may be split in the bile into glycerin (glycerol) and fatty acids which may combine with choline and phosphoric acid to form the phospholipid lecithin.

Lipid Phosphorus (5)— is one of the phosphorus fractions existing in blood in organic combination. Upon extraction of blood with alcohol-ether lipid phosphorus is obtained and is considered in the alcohol-ether organic, soluble class.

Phospholipids (Phosphatides)—is a nitrogenous lipid containing phosphorus which on hydrolysis yields fatty acids, glycerin, and a nitrogenous compound. They are esters of orthophosphoric acid. Lecithin is the best known example.

$H_2C - O - CO - R_1$
$H\ C - O - CO - R_2$
$H_2C - O - P(=O) - O - C_2H_4 - N^+(CH_3)_3$
OH
OH

Alph-lecithin

Lecithin (6)—is a common cell constituent and is considered to play a part in metabolism of fat. Unlike true fats, it contains nitrogen and phosphorus. In the industries, many thousands of pounds of lecithin, obtained from soy-beans are used as an emulsifier and in the manufacture of candies, chocolate, cocoa, margarine, medicines, and even in the dyeing of textiles.

Sterols—are complexed monohydroxy alcohols which are unsaponifiable substances having no close relation to fats. The most important one concerned is *cholesterol*, which exists in the body in two states, free and esterified (combined with fatty acids).

$C_{27}H_{46}O$—cholesterol

Lipotropic agents—are substances having an affinity for fats or oils and thus acting on fat metabolism. Example:

$HO - CH_2 - CH_2 - N^+(CH_3)_3$ (OH)

Choline (ethanol trimethyl ammonium hydroxide).

Choline—is a base comparable to sodium hydroxide. It is important in preventing the accumulation of fat in the liver and is generally regarded as a constituent of the vitamin B complex.

OCCURRENCE OF LIPIDS

Individual members of lipids show large individual variations in solubility, but as a class the lipids are readily distinguishable from the carbohydrates and the proteins, the other two great groups of naturally occurring compounds. They are very wide-spread in nature, being found in all vegetable and animal matter. Some members of this group such as the phosphatides and sterols, are found in all living cells where, with the proteins and carbohydrates, they form an essential part of the colloidal complex of protoplasm. Complex lipids are also found in large quantities in brain and nervous tissues, thus indicating the important role these substances must play in the living organism. Other lipids, such as the fats, and oils, represent the chief form in which excess foodstuffs are stored in the animal body. They arise from ingested lipids and from the metabolism of carbohydrates and proteins, and are stored in fat deposits, such as the subcutaneous connective tissue, the intermuscular connective tissue, the omentum, etc., where they act as heat insulators and as reserve supplies of energy (7).

FUNCTION OF LIPIDS IN GENERAL

Dietary lipids which are ultimately to be metabolized in the body consist for the most part of glycerides of fatty acids. One of the chief functions of dietary fat is to provide a large available reserve of food and essential fatty acids to the organism. Digestion of fats is determined by 1) the normal presence of the digestive fluids in the intestine and 2) an adequate period of time for this digestive process to proceed. Sinclair (8) postulated the following equilibrium as existing in the intestinal wall: fatty acids ⟶ phospholipids ⟶ fat.

There is a specific phospholipid in the intestinal mucosa which occupies an intermediary position between fatty acids and neutral fats. All of plasma phospholipids are the choline type. Therefore, transport of fatty acids by way of phospholipids involve only those phospholipids that contain choline, which is the nitrogenous basic constituent of lecithin. Lecithin is the form in which fat is transported from blood to tissues and in the opposite direction.

While fatty acid in the blood may in part be transported as phospholipids, an important role in this process is also played by the esters of cholesterol and of glycerol (neutral fat) (9).

Protein may play a role in aiding lipid transport in the blood as may be shown that blood lipids are found concentrated in the beta globulin fraction when plasma is fractionated by physico chemical methods (10, 10a). Alteration in total blood lipids are a reflection of changes in the concentration of the individual constituents which comprise the total lipid fraction of the blood.

FUNCTION OF INDIVIDUAL LIPIDS

Cholesterol (11, 12) is an essential constituent of all cells and fluids of the body. It is a precursor of cholic acid and cholestenone and also functions with the bile acids and salts in facilitating the absorption of fatty acids. It is an important means of transportation of fatty acids in the blood, through the formation of cholesterol esters. It is present in highest concentration in the adrenal cortex and is biological precusor of steroid hormones. It is an important source of vitamin D by irriadiation of the skin, with the formation of activated 7-dehydrocholesterol. It may be involved in cell permeability and possibly in immunologic reactions.

SOURCES FOR SERUM CHOLESTEROL

The cholesterol in the body is derived from two sources, that ingested in the diet and that obtained by endogenous synthesis. The only important exogenous dietary sources are certain animal fats and products, including milk, butter, cheese, and egg yolk, and animal organs such as brain and liver; certain sea foods such as oysters are also fairly good sources (13). Endogenous cholesterol has been known to be synthesized in the liver. Gould (14) by studying tissues after administration of radio active carbon compounds has concluded that synthesis of cholesterol takes place also in the intestinal mucosa and skin but not in the aorta.

The liver synthesizes cholesterol at the approximate rate of 0.3 grams per day which is believed to balance the rate at which cholesterol is normally excreted. Cholesterol, whether contained in the diet or produced by the liver, passes through the small intestine. Here, one of two things happens: It is absorbed, or is passed on to the colon and excreted. By reducing absorption, excretion will be increased. Since the fatty acids of dietary fats greatly promote the absorption of cholesterol by converting it to cholesterol esters, a low fat diet would greatly reduce cholesterol absorption and thus greatly aid in its elimination. In addition, a low fat diet also has been reported to increase the excretion of bile acids and since these bile acids are made by the liver from cholesterol, the indirect effect might be to reduce cholesterol.

Exogenous and endogenous cholesterol are present in the intestine and react with the fatty acids. Some free cholesterol is transformed into coprosterol and excreted through the feces. The esterified form is absorbed and is carried to the liver where the cholesterol in part is "liberated," the fatty

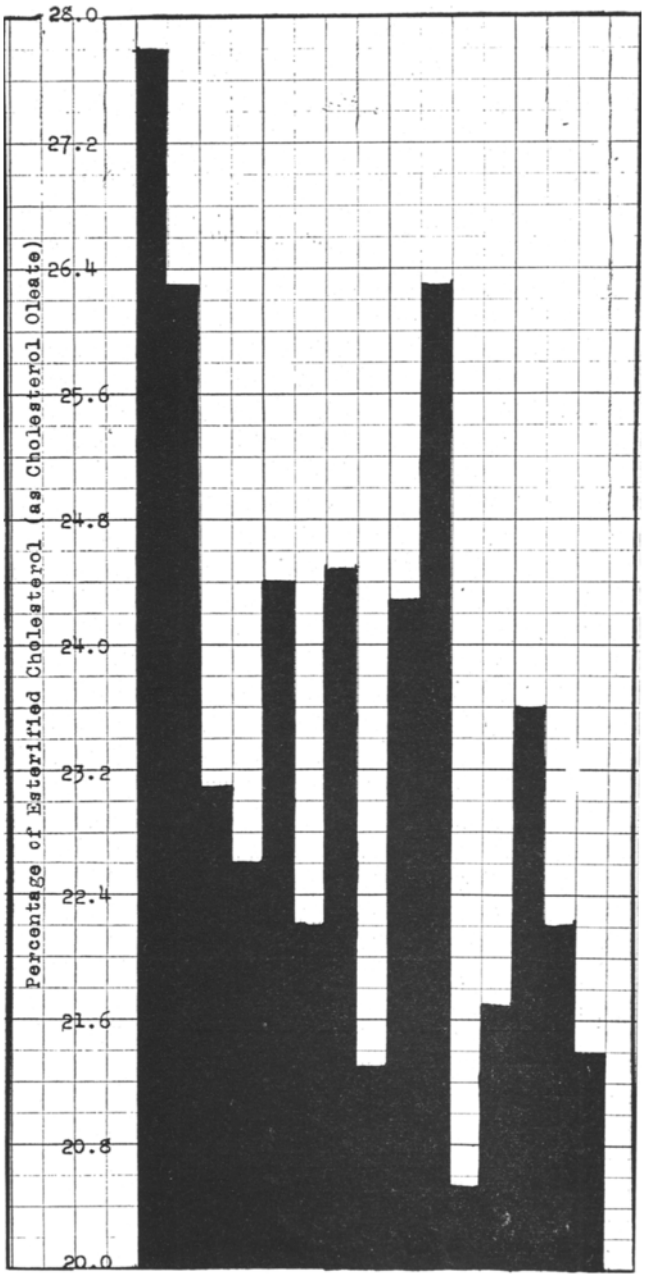

Fig. 3: Percentage of serum esterified cholesterol (as cholesterol oleate) of total lipid concentration in normal individuals.

acids entering into combinations with phosphoric acid and choline, which may be present within the lumen of the intestine to form lecithin. Some fatty acids give rise to acetates, considered to be the building blocks for cholesterol synthesis.

STEROL=CHOLESTEROL ESTER AND ESTERIFIED CHOLESTEROL

By means of hydroxyl group on carbon (3) cholesterol combines with fatty acids to form esters (15). The alcoholic hydroxyl, which can be esterified with different fatty acids by means of an esterase, may be split by reverse action. The esterification of cholesterol changes its physical properties, which not only have an influence on the absorption and transportation of this substance in the tissue fluids but are also important for the composition of the cell lipids. A cholesterol esterase also seems to be present throughout the body. The liver plays an important role in regulating the ratio of free cholesterol to cholesterol esters as it manifests itself in the serum of men and animals; 60-70% of the total cholesterol is normally present as cholesterol esters in the serum.

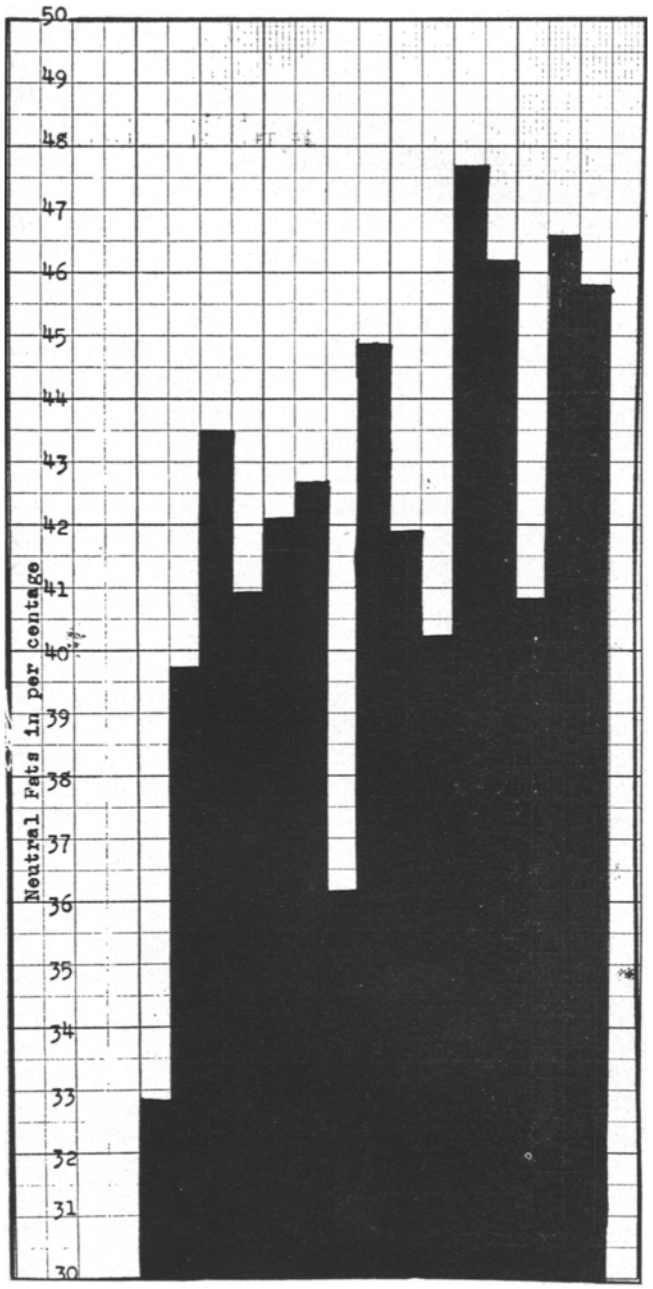

Fig. 4: Percentage of serum neutral fats, in terms of total lipid concentration in normal male and female patients.

Sperry (16) showed that incubation of normal serum increases the ester fraction of cholesterol. He, therefore, assumed that normal blood contains a cholesterol esterase, which may be responsible for maintaining the normal ratio of free cholesterol to cholesterol ester. It is more probable that the cholesterol esterase, which is found in organs, especially the liver, may be responsible for the ratio in the blood. Willibald Klein found that cholesterol esterase in the duodenum originates from pancreatic secretion. He stated also that this esterase is not identical with other esterases (17).

Cholesterol probably enters into complex molecular combination with protein in the parenchyma of the liver. Thus, there are released into the blood stream four varieties of cholesterol, derived fundamentally from either exogenous or endogenous cholesterol.

1. Synthesized (endogenous).
2. Originally ingested (exogenous) now nonesterified.
3. Giant molecular cholesterol-protein complex.
4. Esterified cholesterol (hepatic and intestinal).

Some of this cholesterol is returned to the liver to be secreted in the bile along with cholesterol which enters the bile directly from the liver.

Thus the circuit intestine-liver-blood stream-bile-intestine—the so-called entero-hepatic circulations—is complete.

PHOSPHOLIPIDS AND CHOLINE

Phospholipids are a vital means for transporting fat around the body and for preventing it from infiltrating the arteries, the liver, or other organs. The principal type is lecithin, a substance closely resembling fat in chemical structure, except for this vital difference; it contains choline, linked by phosphoric acid. This choline is a vital substance, a member of the vitamin B complex and specifically recognized as a lipotropic agent, that is, it is essential to keep fat on the move. From choline, supplied by the diet or medicinally, the body can make lecithin and other phospholipids. Liver is the main source of the plasma phospholipids. By radio phosphorus as a tracer, the level of the plasma phospholipids is a measure of the amount of new phospholipid being formed. If the level is high, there is an overproduction of the phospholipid by the liver rather than a piling up due to biliary obstruction or decreased breakdown of the phospholipid molecules by the tissue. This has been found to be true no matter what the cause of the hyperlipemia in all the pathological states studied (18).

The phospholipids are the major form in which fatty acids are transported about the body. There are several kinds of phospholipids: lecithins represent about 50 percent, cephalins about 40 percent, and sphingomyelins about 10 percent of the phospholipids in the blood; the red cells contain even more phospholipids than the plasma, but it is the latter which is believed to be mainly involved in the transportation of fat to the adipose tissues.

In lecithin, the distinguishing chemical entity is choline, while in cephalin it is ethanolamine. The latter is closely akin to choline. Choline is also a component of sphingomyelin.

Animals on a diet free from phospholipids can produce their own from choline. Therefore, choline is looked upon as the lipotropic agent par excellence. From 1 Gm. of choline the body can make 6 Gm. of lecithin, and probably an equal amount of cephalin.

In the ordinary diet choline is present chiefly in the form of phospholipids; since these are fat-soluble substances, they are most plentiful in fats rich in cholesterol, such as eggs, brain, kidney and liver. Consequently, the patient who tries to keep away from cholesterol finds himself automatically cut off from the main sources of choline.

A low fat diet, while it may be poor in choline, need not cause any deficiency of lipotropic factors, provided it be rich in animal proteins, since the latter supply an abundance of methionine, which the body can convert into choline.

Dietary deficiency of choline and other lipotropic factors is known to result in increased production of fat by the liver from carbohydrates. This not only causes fatty infiltration of the liver but also causes the accumulation of cholesterol.

Dietary deficiency of such "lipotropic" substances as lipocaic, methionine, choline, inositol and animal protein causes fatty liver and cirrhosis in animals under certain conditions. The evidence relates these events to a defect in phospholipid synthesis by the liver that accumulates fat in large amounts. Phospholipid synthesis is stimulated by administration of

Case Number	Age	Sex	Height	Weight	Status	Blood Serum Lipids ---- mgm. per 100 ml.										Ratio of phospho-lipids to Cholesterol	Ratio of total cholesterol to phospholipids	Lipid Fraction %				Liver Function Tests							Diagnosis
								Cholesterol										Cholesterol				Thymol Turbidity (units)	Ceph. Floc.						
						Lipid Phosphorus	Phospholipid as lecithin	total	ester as part of cholesterol	% of total	free	% of total	esterified(as cholesterol oleate)	Neutral Fats	Total Lipids			Free	ester(as cholesterol oleate)	phospholipids as lecithin	Neutral Fats		24 hr.	48 hr.	Total proteins	Albumin	Globulin	A/G Ratio (-:1)	
2	42	M	60	116	asthenic short	9.2	230	226	136	60.1	90	39.9	227	268	815	1.01	0.98	11.2	27.8	28.2	32.8	2	0	0	6.94	4.42	2.52	1.7	Psychoneurosis
6	69	M	63	147	sthenic short	8.8	220	190	130	68.4	60	31.6	217	328	825	1.15	0.86	7.4	26.3	26.6	39.7	0	0	0	6.84	4.62	2.22	2.08	Gastritis
8	51	M	65	161	sthenic	9.4	242	196	129	65.8	67	34.2	215	404	928	1.23	0.80	7.4	23.1	26.0	43.5	3	0	0	6.94	4.42	2.52	1.71	Functional dyspepsia
17	51	F	64	120	sthenic	8.4	210	186	108	58.0	78	42.0	180	325	793	1.12	0.87	10.1	22.6	26.4	40.9	2	0	0	7.10	4.74	2.36	2.0	Arthritis
19	41	F	62	181	hyper-sthenic	9.2	230	196	130	66.3	66	33.7	217	374	887	1.17	0.85	7.6	24.4	25.9	42.1	2	0	0	6.84	3.96	2.88	1.30	Psychoneurosis
28	27	F	60	111	asthenic	8.9	222	184	112	60.9	72	39.1	187	359	840	1.20	0.82	8.7	22.2	26.4	42.7	2	0	0	6.48	4.22	2.26	1.86	Psychoneurosis
30	40	M	62	150	sthenic short	7.9	197	193	106	54.9	87	45.1	177	261	722	1.02	0.97	12.2	24.5	27.2	36.1	2	0	0	6.82	4.38	2.44	1.80	Psychoneurosis
31	72	F	58	77	asthenic	8.8	220	200	123	61.5	77	38.5	205	410	912	1.10	0.90	9.7	21.3	24.1	44.9	0	0	0	7.39	4.89	2.50	1.80	Functional dyspepsia
43	44	M	68	163	sthenic	8.4	230	214	134	62.6	80	37.4	223	384	917	1.07	0.93	8.9	24.3	25.0	41.9	3	0	1	7.18	4.13	3.05	1.35	Normal
45	22	F	60	108	sthenic	7.8	195	203	128	63.0	75	37.0	213	325	808	0.96	1.04	9.4	26.3	24.1	40.2	2	0	1	6.84	3.96	2.88	1.30	Psychoneurosis
51	50	F	64	172	hyper-sthenic	9.4	235	200	120	60.0	80	40.0	200	465	974	1.17	0.85	7.8	20.5	24.1	47.7	0	0	0	7.36	4.18	3.18	1.31	Menopause
53	34	F	61	110	sthenic	8.8	220	200	122	61.0	78	39.0	203	431	932	1.10	0.90	8.5	21.7	23.6	46.2	2	0	0	6.68	3.78	2.90	1.30	Normal
56	47	F	64	116	asthenic	7.9	198	202	114	56.4	88	43.6	190	328	804	0.98	1.02	11.0	23.6	24.6	40.8	2	0	0	6.86	4.16	2.70	1.50	Asthenia; hemorrhoids
57	50	M	67	157	sthenic	8.2	205	184	116	60.1	68	39.9	193	407	873	1.11	0.89	7.8	21.8	23.4	46.6	3	0	1	6.49	3.74	2.75	1.36	Normal
62	54	F	67	148	sthenic	8.7	217	192	115	59.8	77	40.1	192	411	897	1.13	0.88	8.7	21.4	24.1	45.8	4	0	1	6.03	3.85	2.18	1.76	Psychoneurosis
Arithmetic Mean			63	136		8.65	218	197	121	61.2	76.2	38.8	202	365	861	1.10	0.90	9.1	23.5	25.3	42.1	2.2	0	.26	6.85	4.23	2.62	1.61	

Table I. Lipid variations of phospholipids, neutral fats, total lipids, lipid fraction percentages and liver function tests in fifteen normal patients, male and female.

choline or of methionine, the latter providing methyl groups for the synthesis of choline. This fundamental work does not specifically indict deficiency of any of these nutrients in the pathogenesis of liver disease in man although, as pointed out above, improper food consumption unquestionably plays an important role. Generally, diets of persons in whom fatty liver and cirrhosis develop, whether associated with alcoholism or not, are, as noted above, deficient in protein, especially that of animal origin. This deficiency includes methionine, choline, and the other nutrients usually classed in the vitamin B complex. The evidence incriminating dietary factors in the pathogenesis of liver disease in animal experiments is necessarily more specific than that in man, but the two bodies of evidence are compatible.

SIGNIFICANCE OF LIPOPROTEIN COMPLEX

Chylomicrons are minute fat globules in which form fat is absorbed from the alimentary tract. The fat contained in the diet enters the blood stream by gateways provided in the small intestines. Some of it escapes the splitting action of the bile and may penetrate directly into the lymphatic outposts, called the lacteals, situated in the intestinal projections known as villi. The rest is principally split into fatty acids and glycerine. Then fatty acids enter into the composition of lecithin and other "phospholipids" and combine with relatively insoluble cholesterol in the bile and in the digestive food itself to form cholesteryl esters which are readily absorbed into the lymph channels. These take the fats and other lipids to the blood stream, where they are suspended in particles about one micron in diameter known as the chylomicrons.

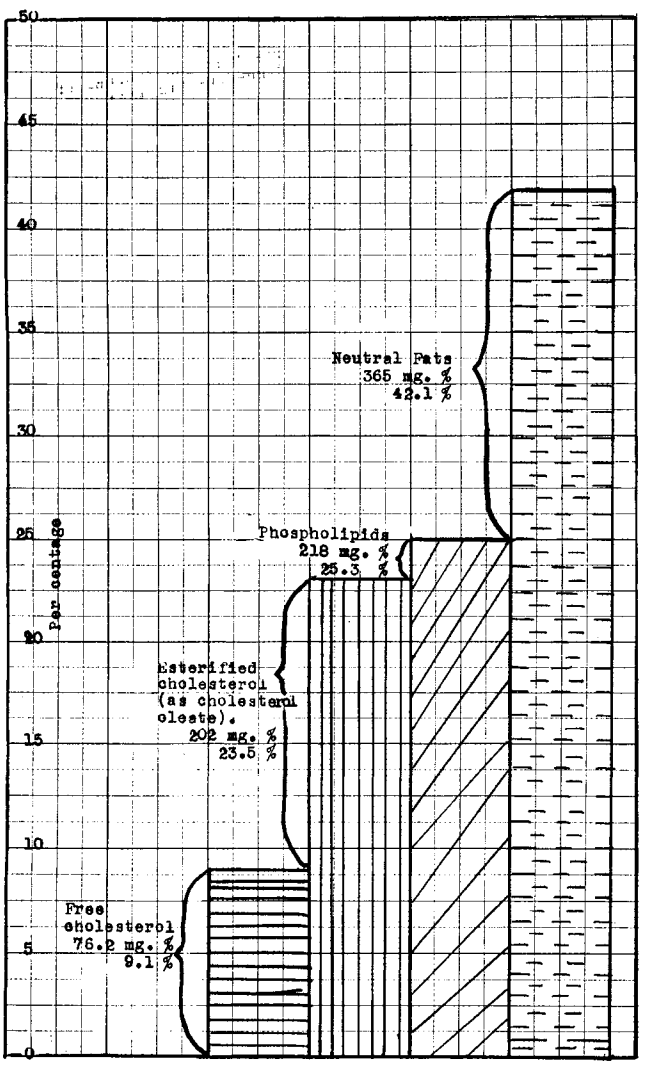

Fig. 5: Graphic representation of arithmetic mean of serum lipid pattern of normal male and female patients. Percentages are in terms of total lipid values. Milligram percent per 100 ml. serum, as determined.

Gofman and his associates (19, 20), by analytical ultracentrifugation, had been able to study quantitatively the entire spectrum of lipid and lipoprotein components of sera. Four classes of chylomicrons have been described on the basis of the Svedberg flotation rates:

Sf 3-8 Normal components of all sera.
Chiefly phospholipids, lipoprotein and cholesterol.
Relatively constant for each individual, vary between individuals. Do not fluctuate with meals or disease.

Sf 10-20 Stable on given diet, not affected by individual meals.
Contain 30 per cent cholesterol.
Possibly related to the occurrence of atherosclerosis.
Sometimes Sf 20-30 are found with the Sf 10-20 group.

Sf 30-70 Major fraction during alimentary lipemia.
Greatly modified by meals.

Sf over 75 Represent the chylomicrons and other large aggregates.
Increased after fat meals and are part of alimentary lipemia.

FUNCTION OF THE LIVER IN FAT METABOLISM

Liver plays an important part in the metabolism of fat as evident by the following facts: (1) The liver extensively desaturates fatty acids (21). The fatty acids in the liver are more unsaturated than the fatty acids found in other tissues (22, 23). Saturation of unsaturated fatty acids has also been shown to occur, producing evidence that saturation and desaturation of fatty acids in the mammalian organism is a physiological reversible process (24). (2) Neutral fat or cholesterol esters may accumulate more in the liver than in

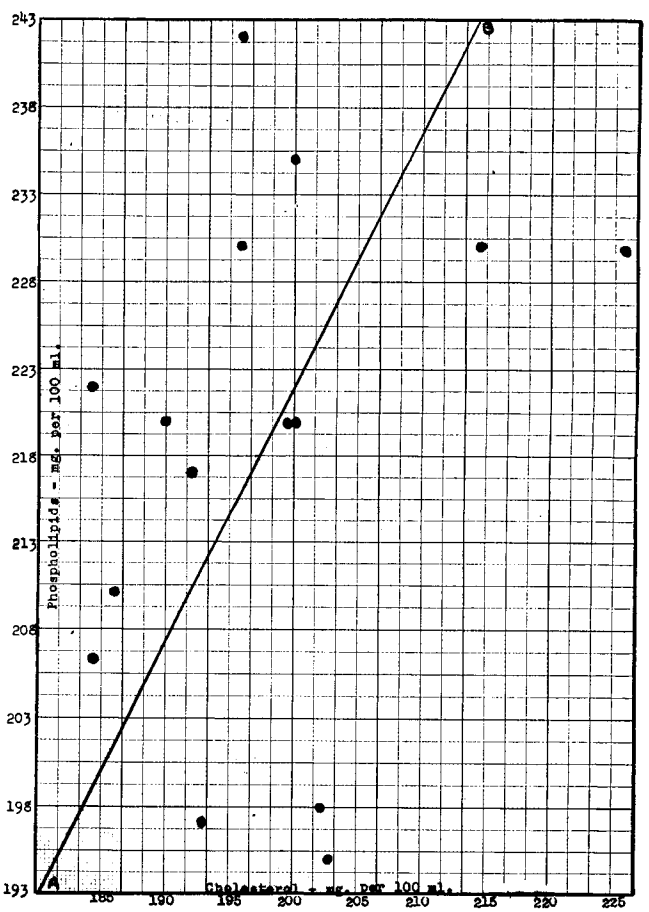

Fig. 6: Scatter graph of relationship between phospholipids and cholesterol in fifteen normal individuals. A-B represents range arithmetic mean.

any other tissue. (3) Ketogenesis occurs chiefly in the liver as a result of the oxidation of fatty acids and is the chief site of formation of ketone bodies (25). (4) The rate of phospholipid turnover in the liver is more rapid than in any other tissue, with the possible exception of the intestinal mucosa during fat absorption. The liver appears to act as a sorting point for fatty acids and a great central laboratory in which phospholipids for the tissues are assembled (26). (5) With respect to steroids, the liver exercises three functions: a) The excretion of cholesterol in the bile; b) The formation and excretion of cholic acid; c) The destruction or inactivation by conjugation of steroid sex hormones. The liver esterifies, synthesizes, stores, and destroys cholesterol (27).

METHOD OF STUDY AND MATERIAL

Patients were chosen who were clinically diagnosed as not suffering from any organic disease especially of the cardio-vascular and coronary systems. The diagnoses were mostly functional dyspepsia and psychoneurosis. No food nor liquids were permitted after the evening meal of the preceding day. Fasting, non-oxalated blood was obtained the following morning, being certain that the patient was at ease, not following any exercise nor effort. Duplicate samples were determined. At least 15 cc. of blood were drawn for lipid fraction and liver studies. The lipid phosphorus (lipid esters of phosphoric acid) was determined by the Youngburg modified digestive method (28). As mentioned above lipid phosphorus was one of the phosphorus fractions existing in the blood in organic combination (5).

It has been shown that the average blood phospholipid contains four percent of phosphorus. Therefore, multiplying by the figure 25 will give the approximation of the weight of the blood phospholipid fraction. Since phospholipids are present in the blood mostly in the form of lecithin, the molecular weight of lecithin is utilized for the determination of phospholipids.

$$\frac{\text{Molecular weight of lecithin (805.7)}}{\text{Atomic wt. of phosphorus (31.2)}} = 25.$$

Total lipids were calculated by the gravimetric modified Bloor method (29).

Total cholesterol and cholesterol esters were determined by the Leiboff method (30). The term cholesterol ester as used clinically is the amount of cholesterol not precipitated by digitoxin and is expressed in terms of cholesterol. In order to express the value of cholesterol ester in terms of actual weight, a factor of 1.67 is utilized. This figure is obtained by dividing the molecular weight of cholesterol oleate by the molecular weight of cholesterol. It is customarily accepted that most of the cholesterol ester of the blood is in the form of cholesterol oleate. This factor of 1.67 is applicable to cholesterol oleate and stearate.

$$\frac{648\ (\text{Wt. of 1 mol. cholesterol} + 1\ \text{mol. oleic acid}\ (-H_2O)}{384\ (\text{Molecular weight of cholesterol})} = 1.67.$$

Cholesterol ester (as cholesterol) $\times$ 1.67 = actual weight of cholesterol ester in mgms. or esterified cholesterol as cholesterol oleate (termed esterified cholesterol by some).

At the present time, there is no practical method to determine neutral fats directly. Neutral fats are ob-

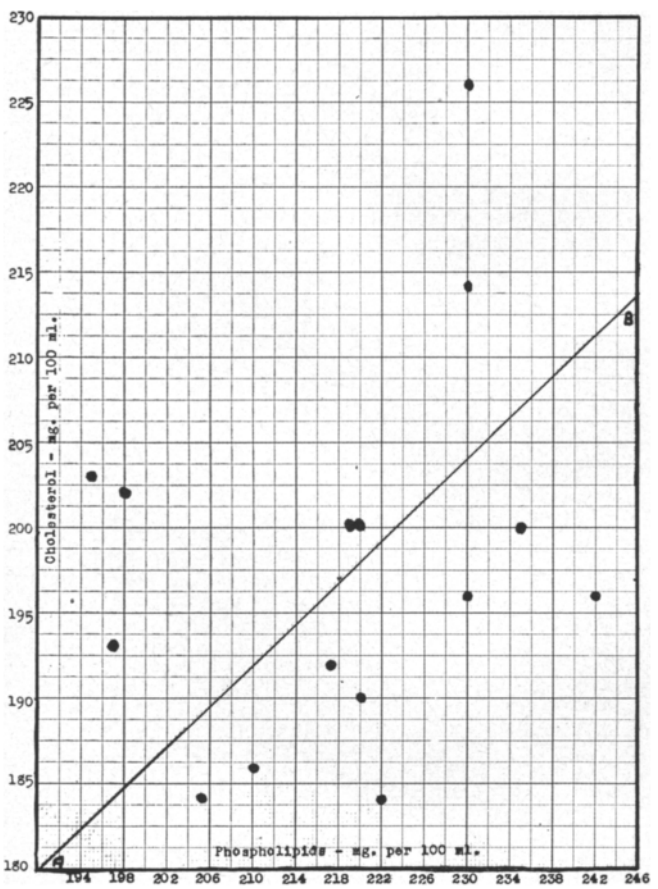

Fig. 7: Scatter diagram of relationship between normal cholesterol and phospholipids. A-B represents range of arithmetic mean.

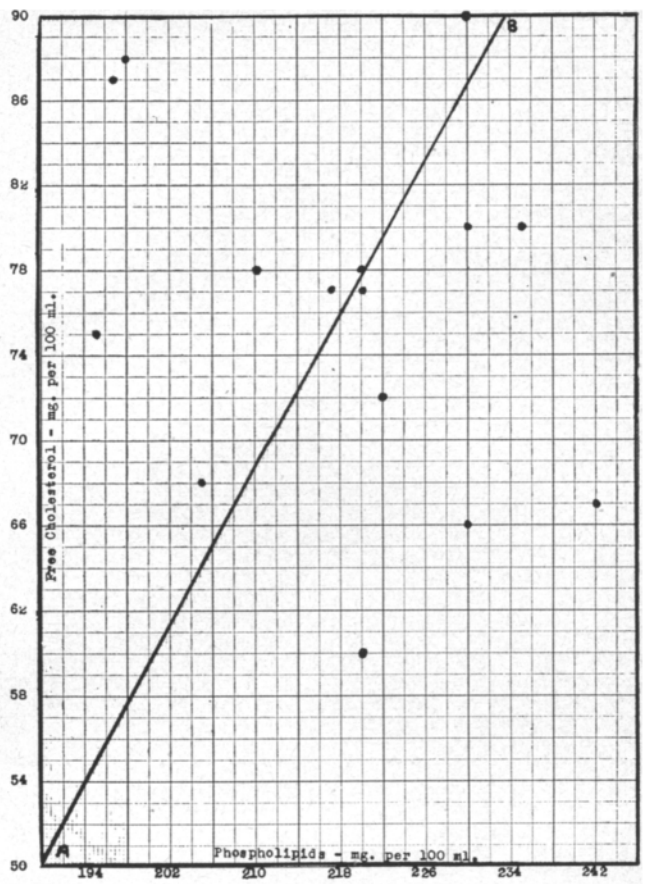

Fig. 8: Scatter graph showing relationship between free cholesterol to phospholipids in normal individuals. A-B represents arithmetic mean.

tained indirectly by subtracting from the total lipid values, the weights of the phospholipid fraction, free cholesterol fraction and the esterified cholesterol as cholesterol oleate fraction.

Lipid fraction percentages were obtained by dividing the weights of the various lipid fractions by the total lipids.

Liver function methods performed were those of the thymol turbidity test of Maclagan (31), cephalin-cholesterol flocculation test of Hanger (32) and the total protein, albumin-globulin values by the Tryrosine method of Greenberg (33). Sedimentation values were performed by the Westergren method (34). Complete blood counts were calculated to exclude any blood disturbance. Height, weight and type of constitution were noted.

RESULTS

Fifteen normal patients were studied as controls, which included nine females and 6 males. The average age was 46 years; height 62.8 inches; and weight 135.8 pounds. Sthenic (normal) type of constitution predominated, as well as the female sex. Table I shows the values of each normal patient, as well as the arithmetic mean for the group. Values varied regardless of the sex, age group, or constitutional status. The highest values for total lipids were found in both the young and the old, male or female and were concomitant with the higher values for lipid phosphorus and phospholipids. The highest total lipid values did not occur concurrently with those of the total cholesterol values and were independent of the high values for neutral fats (Table I). The ratio of the cholesterol esters (as cholesterol) 61.2% and free 38.8% of the total cholesterol values were found to be within normal range.

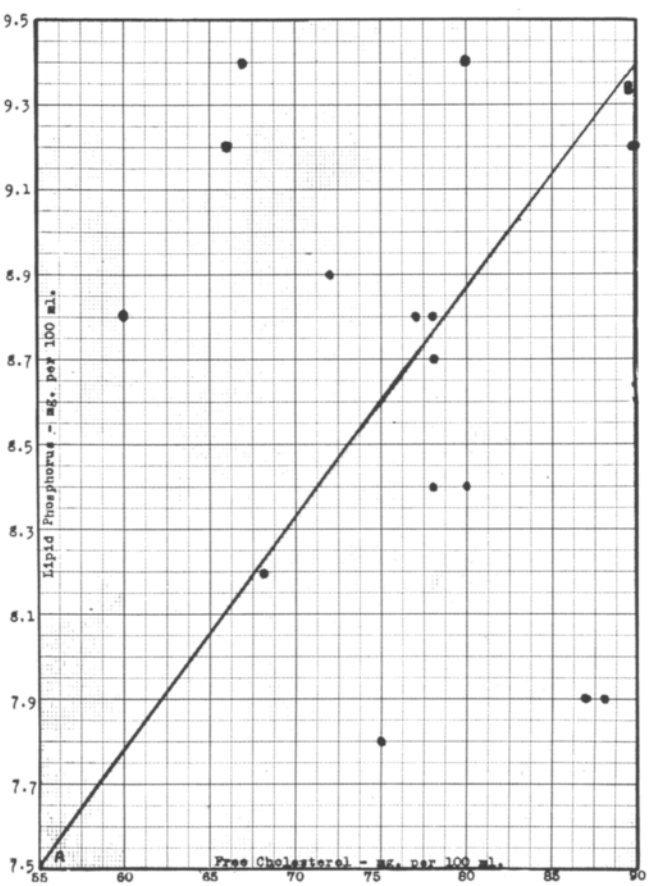

Fig. 9: Scatter graph of lipid phosphorus and free cholesterol in normal male and female patients. A-B represents area of arithmetic mean.

TABLE II

VARIATIONS FROM THE ARITHMETIC MEAN AND RANGE OF THE LIPID FRACTIONS IN MILLIGRAMS PER 100 ML. OF SERUM

Lipid Constituent	Arithmetic Mean	Values Highest	Lowest	Range
Free cholesterol	76	90	60	30
Phospholipids	218	242	195	47
Esterified cholesterol (cholesterol oleate)	202	227	177	50
Neutral Fats	365	465	261	204

Free cholesterol shows the least deviation from the range. Phospholipids and esterified cholesterol reveal practically the same range, while the neutral fats show the highest (see Table II).

TABLE III

VARIATIONS FROM THE ARITHMETIC MEAN AND RANGE OF THE LIPID FRACTIONS IN PERCENTAGES.

Lipid Constituent	Arithmetic Mean	Values Highest	Lowest	Range
Free cholesterol	9.1	12.2	7.4	4.8
Phospholipids	25.3	28.2	23.4	4.8
Esterified cholesterol (cholesterol oleate)	23.5	27.8	20.5	7.3
Neutral Fats	42.1	47.7	36.1	11.6

If the percentage values of the lipid fractions are considered (Table III) the free cholesterol and phospholipids present the same range values (4.8); esterified cholesterol higher (7.3); while the neutral fats are highest (11.6). The variations of the range for neutral fats is much more striking for the molecular weight values, 204 mg. (Table II) than when the percentage values are considered, 11.6 (Table III).

Figures 1, 2, 3 and 4 show the marked normal variations. The graphic representation (figure 1) of free cholesterol appears to vary more than that of neutral fats (figure 4) in spite of the numerical values obtained of the arithmetic mean and range (Tables II and III). Since the graphic representation shows such normal deviation of lipid fractions one must be very cautious to evaluate any therapeutic effects upon abnormal conditions and disease. However, as with any such variation one must compare the "so called" arithmetic mean (average normal) values with those which may be encountered abnormally. The arithmetic mean for lipid fraction percentage is graphically presented in figure 5 showing free cholesterol to be the least (9.1%); esterified cholesterol (23.5%); phospholipids (25.3%). The neutral fats are found to be the highest of the lipid fraction percentage, 42.1% (figure 5). The esterified cholesterol (cholesterol oleate) 23.5% almost approximates the values of phospholipids 25.3%. The free cholesterols were approximately 10% of the total lipid values. The esterified cholesterol (as oleate) and phospholipids constituted about 50% of the lipid values (figure 5). The ratio of the phospholipids to total cholesterol (figure 6) varied more than the ratio of total cholesterol to phospholipids (figure 7). One cannot speak of a constant value for these relationships. It becomes erroneous and dangerous to signal an in-

dividual for atherosclerosis or overt coronary disease because the ratio value of phospholipids to cholesterol or vice versa is above or below 1.00 (35, 36, 37). Even the ratio of free cholesterol to phospholipids (37) (figure 8), or lipid phosphorus to free cholesterol (figure 9), or lipid phosphorus to total cholesterol (figure 10) are not as constant as some report (38).

Thymol turbidity and cephalin-cholesterol flocculation tests showed no abnormal values. The total protein values, with the albumin and globulin partitions as well as the albumin-globulin ratios were within normal range (Table I). The above laboratory data definitely excluded liver pathology and hypoproteinemia.

DISCUSSION

Both the lay and medical publications have been on the increase recently as to atherosclerosis, coronary artery disease, and the values of blood lipid studies. Patients have become so cognizant as to request "blood fat studies." Experimental work has been done producing lesions of atherosclerosis akin to that found in humans by chronic cholesterol feeding.

Numerous publications have mentioned the values of cholesterol and phospholipids, in both normal and abnormal conditions, whether with or without therapy (39, 40, 41, 42, 43). It was felt that normal individuals should first be studied to ascertain the constancy and variations of the blood lipid values. It is important that such determinations be made so as to obtain a base line for comparison.

The results for normals showed the female sex predominated as well as the normal sthenic habitus with no visible influence upon lipid values. One of the highest lipid values (912 mgm. percent) was found in an underweight patient with an asthenic habitus (case No. 31). The average value for total lipid was encountered in a well built individual with a hypersthenic constitution. The ratio of phospholipids to cholesterol was found to be more than 1.00 in a 72 year old female (case No. 31); surely some atherosclerosis must have been present in such an aged individual. If one were to rely on a ratio value of phospholipids to cholesterol of less than 1.00 as being characteristically found in atherosclerosis, one would be misled. The patient (case No. 31) age 72 showed a phospholipid to cholesterol ratio of 1.10, which many observers maintain is found in non-atherosclerotic individuals. If malnutrition were to play a role, a value of less than 1.00 should be obtained in such an underweight person as this 72 year old female (case No. 31).

On the other hand, the lowest ratio of phospholipids to cholesterol (0.96) was found in a young female, age 22 (case No. 45). While it would be exceptional and rare to find overt atherosclerosis in such a young individual, yet such a condition would be considered by some to be present, if the ratio of phospholipids to cholesterol is less than 1.00.

However, one must state that the ratio of phospholipid to cholesterol is 1.00 and above in normals (13 out of 15 patients—86%). But, one cannot deny that some atherosclerosis must be present in some of these normal patients studied as controls, ranging from 50 to 72 years of age. Is it correct to conclude because the phospholipid to cholesterol ratio is 1.00 and above, that no atherosclerotic process is present?

The ratio of cholesterol to phospholipid was found to be less than 1.00 in the same number of instances, 13 out of 15 (86%). The arithmetic mean for the phospholipid to cholesterol ratio was found to be 1.10; while that for cholesterol to phospholipid 0.96. The larger phospholipid to cholesterol ratio occurred where the lipid phosphorus or phospholipid values were comparatively higher than the highest total lipid findings. The higher cholesterol to phospholipid ratio was found where the phospholipid values were lower and independent of the highest total lipid findings.

Complete blood counts excluded any latent or possible haematological disturbances. Liver function tests as mentioned above under methods, excluded any liver pathology, especially protein dysfunction and hypoproteinemia.

Lipid constituents commonly encountered and readily analyzed without any complicated procedures were determined, such as lipid phosphorus, total lipids, total cholesterol and cholesterol esters as cholesterol. The phospholipid values were calculated from the lipid phosphorus values and the neutral fats, as mentioned under methods. It has been shown without any question or any doubt that total cholesterol values do not bespeak for any increased or decreased total blood lipid values. It becomes essential to consider these varied lipid components if we should speak of any such disturbance. Values for phospholipids and cholesterol also are not sufficient to reflect the total fat or lipid constituents of blood. All of these lipid fractions may vary. While it is true that the ratio of the phospholipid to total cholesterol or vice versa are more constant than

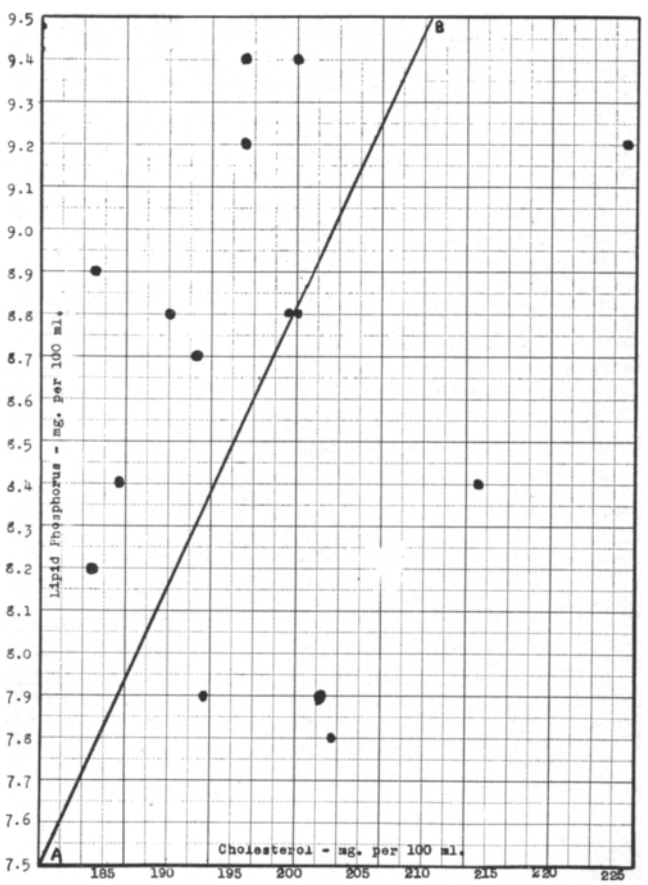

Fig. 10: Scatter diagram of relationship between lipid phosphorus and cholesterol in normals. A-B—region of arithmetic mean.

the individual lipid constituents, yet one may encounter a ratio in normal persons that some may consider characteristic for atherosclerosis or coronary artery disease. One must use caution and discretion as shown above in cases No. 31 and No. 45. Some objections may be raised that the values herein reported are not scientifically accurate since many determinations over a prolonged period may show variant results. While this may be true, yet it is essential as clinicians for us to know the limitations to be employed for clinical practice. Patients will not withstand such extravagance, drawn out laboratory procedures, and time. It is important therefore for practicing physicians to draw the line; utilize his arithmetic mean values, and proceed therefrom.

Animal experimentation and prolonged laboratory work cannot be carried on ad infinitum in clinical practice. Differences in values of the concentrations of lipids in the blood may arise from difference in analytical technique; unreliability of certain methods employed, and the terminology of the constituents such as designating total fatty acids as "fats and neutral fats" or neutral fats as "total fatty acids" (44).

SUMMARY

1. Fifteen normal patients were studied as controls including 9 female and 6 male. The age varied from 22 to 72, with an average of 46 years. Constitutional types were 9 sthenic; 4 asthenic; and 2 hypersthenic.

2. Serum lipid studies included lipid phosphorus, phospholipids (twenty-five times the value of lipid phosphorus), total cholesterol, cholesterol ester, and total lipids. The values for esterified cholesterol (as cholesterol oleate) were obtained by multiplying the cholesterol ester values by the factor 1.67.

3. Liver disturbances were excluded by the liver function tests of thymol turbidity; cephalin-cholesterol flocculation; total protein, albumin and globulin ratios.

4. Haematological disturbance was excluded by complete blood count determinations and inflammatory processes by the sedimentation rate.

5. Neither sex, constitutional status, nor age had any effect upon normal blood lipid values.

6. There was marked variation of each lipid fraction percentage of cholesterol (free and ester as oleate), phospholipids, and neutral fats. Addition of these values corresponded to total lipids determined. Cholesterol oleate (esterified) and neutral fats showed the greatest variations.

7. Total cholesterol values are not indicative of total blood lipid elevations.

8. Neutral fat values are independent of high or low lipid phosphorus, phospholipid or cholesterol values. Ratios of phospholipids to cholesterol did not speak for the higher neutral fat percentage. Higher neutral fat percentage values may be found in some normal cases of low phospholipid-cholesterol ratios.

9. The ratio of phospholipid to total cholesterol or vice versa may vary and cannot be considered absolutely suggestive of atherosclerosis or coronary artery disease. High ratio values above 1.00 of phospholipid to cholesterol may be found in older individuals, while ratios of phospholipids to cholesterol below 1.00 may be encountered in the young age group. The ratio of phospholipid to cholesterol may not be a mirror image of the highest blood lipid values. While higher phospholipid cholesterol ratios may be due to lesser cholesterol values rather than higher total blood lipid findings, yet generally the phospholipid values correspond to the higher blood lipid findings.

10. The ratio of cholesterol to phospholipid was found below 1.00 in some normal patients. The ratio of free cholesterol to phospholipid or vice versa was not found to be more constant than the ratio of phospholipid to cholesterol.

CONCLUSIONS

1. Serum lipid fractions as lipid phosphorus, phospholipids, cholesterol, cholesterol as oleate (esterified), neutral fats, and total lipids were defined and determined in normal male and female patients, twenty-two to seventy-two years of age. These serum lipid fractions must be determined to reflect the true blood lipid state.

2. There is a marked variation from the arithmetic mean of the normal serum lipid fractions.

3. Neither total cholesterol alone nor cholesterol and phospholipid determinations reflect high blood lipid changes.

4. The ratio of phospholipid to cholesterol above 1.00 may be encountered in older individuals and below 1.00 in the young age group.

5. One must determine the arithmetic mean values as a base line, because of the variations of the normal serum lipid fraction percentage; the difference in technique; and the variation in the terminology of certain blood lipid constituents.

REFERENCES

1. Peters, John P. and van Slyke, Donald D.: Quantitative Clinical Chemistry. Interpretations. Vol. I. Williams and Wilkins Co., Balt.; 2nd ed.; p. 374, 1946.
2. Bloor, W. R.: Biochemistry of the Fatty Acids and their Compounds, the Lipids. Reinhold Publishing Co., New York; p. 1, 1943.
3. Harrow, Benjamin: A Textbook of Biochemistry. W. B. Saunders Co., Phil.; p. 110, 1950.
4. Thannhauser, Siegfried J.: Lipidoses. Diseases of the Cellular Lipid Metabolism. Oxford University Press, New York; 2nd ed.; p. 11, 1950.
5. Best, Charles H. and Taylor, Norman B.: The Physiological Basis of Medical Practice. Williams and Wilkins Co., Balt.; 5th ed.; p. 4, 1950.
6. Harrow, Benjamin: A Textbook of Biochemistry. W. B. Saunders Co.; Phil.; p. 122, 1950.
7. Hawk, Philip B.; Oser, Bernard L.; and Summerson, William H.: Practical Physiological Chemistry. Blakiston Co., Phila.; 12th ed.; p. 86-87, 1947.
8. Sinclair, R. G.: Role of Phospholipids of Intestinal Mucosa in Fat Absorption, with Additional Data on Phospholipids of Liver and Stomach and Skeletal Muscle. J. Biol. Chem.; 82:117-136, 1929.
9. Duncan, Garfield G.: Diseases of Metabolism. W. B. Saunders Co., Phil.; 2nd ed.; p. 162-167, 1947.
10. Blix, G.: Electrophoresis of Lipid-Free Blood Serum. J. Biol. Chem.; 137:495, 1941.
10a. Fishberg, A. M.; Friedfeld, L.; Hoffman, I.; Stoller, E. R.; and Fishberg, E. H.: Beta-Hyperglobulinemia Produced by Cholesterol Feeding in the Rabbit. Proc. Soc. for Exper. Biol. and Med.; V. 75, 301-303, 1950.
11. Bloch, K.: The Intermediary Metabolism of Cholesterol. Circulation; 1:214, (Feb.) 1950.
12. Gubner, R. and Ungerleider, H. E.: Arteriosclerosis. A Statement of the Problem. Am. J. Med.; 6:60 (Jan.) 1949.

13. Turner, D.: Handbook of Diet Therapy. Univ. of Chicago Press, Chicago; p. 79, 80; 1946.
14. Gould, R. G.: The Comparative Metabolism of Dietary and Endogenous Cholesterol Differentiated by the use of Radioactive Carbon. Circulation; 2: 467, (Sept.) 1950.
15. Thannhauser, Siegfried J.: Lipidoses, Diseases of the Cellular Lipid Metabolism. Oxford University Press, New York, 2nd ed.; p. 37, 1950.
16. Sperry, W. M.: Cholesterol-esterase in Blood. Jour. Biol. Chem.; CXI, 467, 1935.
17. Klein, Willibald: Über die enzymatische Hydrolyse der Cholesterinester. Zeitsch. f. Physiol. Chem.; CCLIX, 268, 1939.
18. Balfour, William M.: Human Plasma Phospholipid Formation: A Study made with the aid of Radiophosphorus. Gastroent.; 9: 686-694, 1947.
19. Gofman, J. W.: The Role of Lipids and Lipoproteins in Atherosclerosis. Science; 111:166, 1950.
20. Gofman, J. W.; Jones, H. B.; Lindgren, F. T.; Lyon, T. P.; Elliott, H. A.: and Strisower, B.: Blood Lipids and Human Atherosclerosis. Circulation; 2:161 (Aug.) 1950.
21. Leathes, J. B. and Raper, H. S.: The Fats. Monographs on Biochemistry. Longmans Green and Co., London; 2nd ed.; 1925.
22. Page, I. H.; Kirk, E.; Lewis, W. H., Jr.; Thompson, W. R. and van Slyke, D. D.: Plasma Lipids of Normal Men at Different Ages. J. Biol. Chem.; 111, 613, 1935.
23. Schoenheimer, R. and Rittenberg, D.: Deuterium as an Indicator in the Study of Intermediary Metabolism. V. The Desaturation of Fatty Acids in the Organism. J. Biol. Chem.; 113:505, 1936.
24. Rittenberg, D. and Schoenheimer, D.: Deuterium as an Indicator in the Study of Intermediary Metabolism. VIII. Hydrogenation of Fatty Acids in the Animal Organism. J. Biol. Chem.; 117:485, 1937.
25. Mirsky, I. A.: The Source of the Blood Acetone Resulting from the administration of the Ketogenic Principles of the Anterior Hypophysis. Am. J. Physiol.; 115: 424, 1936.
26. Peters, John P. and van Slyke, Donald D.: Quantitative Clinical Chemistry. Interpretations. Williams and Wilkins Co., Balt.; V. I, 2nd ed.; p. 423, 1946.
27. Idem. p. 456.
28. Youngburg, G. E. and Youngburg, M. V.: Phosphorus Metabolism: System of Blood Phosphorus Analysis. J. Lab. and Clin. Med.; 16: 158-166, (Nov.) 1930.
29. Bloor, W. R.: A Method for the Determination of Fat in Small Amounts of Blood. J. Biol. Chem.; 17:377, 1914.
30. Leiboff, S. L.: A Simplified Method for Cholesterol Determination in Blood. J. Biol. Chem.; 61, 177, 1924.
31. Maclagan, N. F.: Serum Colloidal Gold Reaction as Liver Function Test. Brit. J. Exper. Path.; 25: 15-20, 1944.
32. Hanger, F. M.: Serological Differentiation of Obstructive from Hepatogenous Jaundice by Flocculation of Cephalin-Cholesterol Emulsions. J. Clin. Investigation; 18:261-269, (May) 1939.
33. Greenberg, D. M.: Colorimetric Determination of Serum Proteins. J. Biol. Chem.; 82:545, 1929.
34. Westergren, A.: Suspension stability in Pulmonary Tuberculosis. Acta med. Scandinav.; 54:247, 1921.
35. Kellner, A.; Correll, J. W. and Ladd, A. T.: Modification of Experimental atherosclerosis by means of Intravenous Detergents. Am. Heart J.; 38:460, 1949.
36. Ahrens, E. H., Jr. and Kunkel, H. G.: Stabilization of Serum Lipid Emulsions by Serum Phospholipids; J. Exper. Med.; 90:409, 1949.
37. Morrison, Lester M.; Gonzalez, P. and Wolfson, E.: The Phospholipid Cholesterol Ratio as a Test for Atherosclerosis. Circulation; II:3 p. 472, (Sept.) 1950.
38. Albrick, Margaret J.; Man, Evelyn B. and Peters, John P.; Serum Lipids in Infectious Hepatitis and Obstructive Jaundice. J. Clin. Invest.; 29:781, (June) 1950.
39. Jackson, Raymond S.; Wilkinson, Jr., Charles F.; Hand, Eugene A.; Waldron, A. M. and Vogel, William C.: The Relationship between the Phospholipids and the Cholesterol in Human Plasma. Circulation, II, 3:472, (Sept.) 1950.
40. Davis D.; Stern, B. and Lesnick, G.: The Lipid and Cholesterol Content of the Blood of Patients with Angina Pectoris and Arteriosclerosis. Ann. Int. Med.; 11:354-369, 1937.
41. Steiner, Alfred: Cholesterol in Arteriosclerosis, with special reference to Coronary Arteriosclerosis. Med. Clinics of North America; 34:673, (May) 1950.
42. Gertler, M. M.; Garn, S. M. and Lerman, J.: The Interrelationships of Serum Cholesterol, Cholesterol Esters and Phospholipids in Health and Coronary Artery Disease. Circulation; 2:205-214, 1950.
43. Watkin, Donald M.; Froeb, Herman F.; Hatch, Frederick T. and Gutman, Alexander B.: Effects of Diet in Essential Hypertension. II. Results with Unmodified Kempner Rice Diet in Fifty Hospitalized Patients. Am. J. Med.; IX, 4:442-493, (Oct.) 1950.
44. Kornerup, Valdemar: Concentrations of Cholesterol, Total Fat and Phospholipid in Serum of Normal Man. Arch. Int. Med.; 85:3, p. 398-415, (March) 1950.

SIGMOIDO-RECTAL INTUSSUSCEPTION AND THE UNSTABLE COLON*

EMIL GRANET, M. D. New York, N. Y.

A CONSIDERABLE portion of patients seen in private or clinic practice complain of disorder of colon function. Symptoms have to do mostly with disturbances of the excretory function. These may express themselves by decreased activity of the bowel under sympathetic stimulation which results in dehydrated and less frequent evacuations, i. e., chronic constipation. Chronic non-inflammatory diarrhea, the passage of excess flatus and mucus and constipation alternating with diarrhea are less frequent manifestations of increased secretion and motility of the large bowel acting under the influence of parasympathetic stimulation. The inclusive term, "unstable colon" was introduced by Kantor (1) to apply collectively to these disturbances.

Chronic constipation has been classified as "colonic" and "rectal." Colonic constipation results from inadequacy of purposeful caudad peristalsis in the large bowel; it is most often spastic though the atonic form follows abdominal surgery, pregnancy and severe trauma.

Rectal constipation denotes a failure of the rectum to empty itself. Hurst termed this type of difficult defecation "dyschezia." It most commonly results from the loss of sensitivity to the defecation reflex, the "call of nature." Repeated "holding back" to suit the hurried pattern of contemporary urban life soon results in increased passive tolerance of the normally empty rectum to mechanical distention by the fecal mass.

*From the Surgical Service (Proctology), French Hospital, N. Y. C.

Submitted July 12, 1951.