EFFECTS OF THE STRUCTURAL ARRANGEMENT OF DIETARY TRIGLYCERIDES ON THE LIPID COMPOSITION OF RAT TISSUES

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Charles Edward Elson

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THESIS

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ABSTRACT

EFFECTS OF THE STRUCTURAL ARRANGEMENT OF DIETARY TRIGLYCERIDES ON THE LIPID COMPOSITION OF RAT TISSUES

by Charles Edward Elson

In order to determine the effects of dietary lipid structure on lipid metabolism in the rat, a lipid fraction containing 33 percent polyunsaturated fatty acids at the α-ester position was isolated from lard (diet B). A second lipid containing an equal amount of polyunsaturated fatty acids esterified at the β-position was isolated from soybean oil (diet C). The effects of these diets on rat feces, subcutaneous, perirenal, blood serum, liver and aorta lipids were compared to those of an iso-caloric, fat-free diet (diet A). Three groups of four mature males were maintained on these diets for a two week period.

Liver and blood serum free and total cholesterol were determined colorimetrically. Gravimetric procedures were used to determine total lipid, neutral lipid and phospholipid content of these tissues. The gas chromatograph was used to identify the methyl esters of fatty acids from phospholipids and each neutral lipid fraction which had been separated by thin-layer chromatographic procedures.

When dietary lipids supplied 40 percent of the calories, feces and blood serum lipid levels were elevated. The polyunsaturated fatty acid (PUFA) content of feces produced by diet C was slightly elevated. All diets produced highly unsaturated body fats. The liver and blood cholesterol levels were lowered by feeding diet C, this decrease occurring in the free cholesterol fraction. Liver phospholipid and triglyceride PUFA patterns were quite similar in the absence of dietary effects. In

blood serum, the phospholipids differed significantly in their composition as compared to the neutral lipids. It was generally found that the PUFA content of the liver and aorta lipids was quite similar while the blood lipids contained significantly lower amounts of PUFA.

A possible mechanism for the blood serum cholesterol lowering effects of vegetable oils, based on these experimental observations and conclusions of other investigators is suggested.

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By

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INTRODUCTION

Research in the general area of public health over the past 15 years has greatly influenced today's diet, especially the lipid components of the diet. This research has generally indicated that the substitution of vegetable oils for animal fats, the addition of linoleic acid to the diet, or the reduction of fat intake would lower serum cholesterol levels. High serum cholesterol levels have, on the basis of extensive population studies, been associated with coronary thrombosis susceptibility. Naturally, this work has had a vast influence on present dietary patterns. The food industry has seen a steady decrease in the consumption of animal fats and an increase in the consumption of fats from vegetable sources. New products have been developed and widely advertised on the basis of their polyunsaturated fatty acid content. New markets have been created for agricultural products such as safflower seed while other markets are being drastically reduced.

In view of the economic effects and perhaps, healthful effects, of this research, the present study was initiated to determine if the polyunsaturated fatty acid content of vegetable oils is the sole factor responsible for the cholesterol lowering effects.

Two fats were selected, each containing 33 percent polyunsaturated fatty acids. One of the fats was from a vegetable source and contained the unsaturated fatty acids primarily at the β -position of the triglyceride molecule. The other was from an animal source and its unsaturated fatty acids were primarily esterified at the α -position. These fats were

introduced into rat diets in equal amounts.

The effects of these diets were compared with those of an isocaloric, fat free diet in order to study their effects on blood serum cholesterol levels, liver cholesterol levels, and the fatty acid composition of lipid fractions isolated from blood, liver, aorta, lipid pools and feces.

REVIEW OF LITERATURE

Weinhouse and Hirsch (1940) described atherosclerosis or atheroma as essentially a focal and predominantly an intimal change and should not be confused with generalized arteriosclerosis or other circulatory disorders. Since the essential components of the human atheromatous plaque appear to be intimal fibrous and lipid infiltration, it is therefore not unnatural that most theories on its pathogenesis center around either fat, fibrin or both, but considerable controversy exists as to which component is primary and which is secondary.

The filtration theory, as described by Page (1954) implies that lipid infiltration of the intima is the primary process and that the fibrous tissue present indicates the reaction to the presence of fat. This theory postulates that the centrifugal force of the circulation drives fat into contact with arterial intima. Over the years, lipid deposits accumulate, but this is accelerated or intensified in the presence of blood lipids in excessive amounts or abnormal forms, if the intraluminar or lateral filtration pressure is high or if the endothelium of the vessel walls is abnormally permeable. According to Weinhouse and Hirsch (1940), and Rabinowitz (1960), a satisfactory explanation is at hand for the particular localization of the plaques at sites of pressure or velocity change within the circulatory system, and for the increased severity of atherosclerosis in the presence of hypertension and such disorders of lipid metabolism as diabetes mellitus and essential xanthomatosis. The steady encroachment of the plaque on the lumen of the vessel favors

super-added thrombosis from circulatory stasis. They stated that cholesterol is present in high concentrations in the lipids of the atheromatous plaques, the chemical nature of which resembles that of blood plasma.

Swell et al. (1960b) reported that in humans with coronary artery disease, the media and serum had approximately the same cholesterol esterified fatty acid composition. Linoleic acid accounts for 40 percent of the total fatty acids. The thickened intima and the plaques had nearly the same cholesterol esterified fatty acid composition and contained significantly less linoleic and arachidonic acids and more oleic acid and saturated fatty acids. Palmitic and oleic acids comprised from 72 to 75 percent of the total fatty acids in the triglyceride fraction. The percentages of palmitic and oleic acids were strikingly constant and were within the ranges of 29 to 35 percent and 39 to 46 percent, respectively. A relatively high level of stearic acid, 11.1 percent, was found in the media triglyceride fraction. A much lower level of polyunsaturated fatty acids was found in the media triglyceride fraction than in the esterified cholesterol fraction.

Swell et al. (1960b) also reported that the phospholipid fraction was characterized by a high level of saturated fatty acids and significantly more arachidonic acid. Palmitic, stearic, and oleic acids accounted for 50 to 59 percent of the total fatty acids.

In another report, Swell et al. (1960a) found that the composition of cholesterol esterified fatty acids of aortic media was very similar to that of serum in elderly humans with atherosclerosis. The plaque material contained a greater proportion of saturated and monoenoic acids than did

the serum or the media. In the media and serum, the major cholesterol ester contained linoleic acid while in the plaques and liver, cholesterol was primarily esterified with oleic acid.

Evrard et al. (1962) found dissimilarities between the cholesterol esterified fatty acid patterns of plasma and atheroma. They concluded that this excludes the likelihood that the cholesterol esters accumulate in the aortic intima by a simple random deposition of the plasma cholesterol esters. Swell et al. (1962) stated that the aortic cholesterol esters were not of constant composition but were dependent upon the dietary fat. They suggested that derangements of cholesterol oleate metabolism may be important factors in the deposition of cholesterol esters in the aortas and liver. Mukherjee et al. (1958) stated that the effects of feeding different dietary fats on rat liver cholesterol levels were apparent within one weeks time.

Swell et al. (1960a) indicated that the serum and liver of normal rats were distinctly different in their cholesterol esterified fatty acid composition. Swell et al. (1960c) found that up to 70 percent of the total cholesterol esterified fatty acids in the serum were unsaturated, the main serum cholesterol esterified fatty acid being arachidonic acid. The major fatty acids of the liver cholesterol esters were of the saturated and monoenoic type with palmitic acid predominating (32.5 percent). They concluded that liver and lymph cholesterol esters play an important regulatory role in blood cholesterol level and composition.

Klein (1958) and Avigan and Steinberg (1958) had previously reported that rat plasma and liver cholesterol esters differ markedly in fatty

acid composition. The polyunsaturated fatty acid composition of the plasma cholesterol esters was much higher than in the liver esters. Also, the cholesterol levels did not follow the same relationship to dietary fats. The liver cholesterol concentration was found to be higher when diets contained a higher proportion of unsaturated fatty acids while the inverse was true of the blood plasma. The work of Russell et al. (1962) with corn oil and lard substantiated these results. On diets with 10 percent corn oil or lard, rat livers contained 3.33 and 2.65 mg cholesterol per gram, respectively, while the cholesterol levels in the blood serum were 71 and 110 mg percent. When the dietary fat was increased to 30 percent, the corn oil diet produced 4.37 mg/g and 67 mg percent while the lard diet produced 3.23 mg/g and 100 mg percent in the liver and blood serum. Reiser et al. (1963) also were in agreement that diets high in unsaturated fatty acids produced higher cholesterol levels in rat livers. On a low fat diet, the liver cholesterol level was only 2.3 mg/g, saturated triglycerides produced 4 and 5 mg/g and trilinolein and safflower oil diets, 7.3 and 7.8 mg/g, respectively. They stated that these responses are compatible with the observed increases in fecal steroids which accompany decreases in serum cholesterol caused by unsaturated dietary fat, since the major route of cholesterol from serum to intestine, as cholesterol and bile acids, passes through the liver. They believed an increase in liver cholesterol levels on unsaturated fat diets supports the hypothesis that the mechanics by which unsaturated acids maintain lower serum cholesterol is by their influence on transport, either by forming labile esters, or by forming unsaturated phosphatides which may

aid in the transport of cholesterol esters across cell membranes. Gerson et al. (1961) were in complete agreement.

Okey et al. (1957) and Linazasoro et al. (1958) were unable to find differences between diets on liver cholesterol levels. Aftergood et al. (1956) reported that rats fed 15 percent lard diets had higher liver cholesterol levels than those fed 15 percent cottonseed oil diets. Their reasoning was that possibly the difference in action of the two fats was due to the greater quantity of essential fatty acids found in cottonseed oil over that which occurs in lard. These essential fatty acids may be required for normal cholesterol transport and metabolism; an inadequate supply will tend to cause an accumulation of cholesterol in the liver. Alfin-Slater et al. (1954) stated that the cholesterol concentration in the liver of rats on low essential fatty acid diets were increased. This increase over the liver cholesterol levels in rats fed 12.5 percent corn oil occurred almost exclusively in the ester fraction. The rats had free cholesterol levels of 1.75 and 1.85 mg/g and cholesterol ester levels of 0.31 mg/g and 1.30 mg/g on 12.5 percent corn oil and fat free diets, respectively. They concluded that cholesterol esters with saturated fatty acids may be unavailable for proper metabolism and transport.

Mukherjee and Alfin-Slater (1958) also reported that diets deficient in essential fatty acids resulted in an increased cholesterol level in rat livers. This accumulation of liver cholesterol induced a decrease in cholesterol synthesis. When essential fatty acids were added to the diet, liver cholesterol levels returned to normal and cholesterol synthesis increased.

Reiser et al. (1960) reported that miniature pig plasma and liver cholesterol esters were comparable as to fatty acid composition. Karmen et al. (1963) may be in agreement since they reported that in humans the cholesterol esterified fatty acids were primarily of endogenous origin and that the esters showed a marked specificity for oleic acid.

On the other hand, Swell et al. (1964) reported that the cholesterol esters synthesized in rat liver microsomes bear no relationship to the cholesterol esters present in rat serum. They believed that it is possible that only certain esters are released, to an appreciable extent, into the blood.

In fasted rats, Swell et al. (1960c) reported that cholesterol esters from serum and liver had 12.4 and 32.5 percent palmitic acid, 2.6 and 21.9 percent stearic acid, 9.8 and 20.1 percent oleic acid, 19.5 and 12.2 percent linoleic acid and 50.0 and 8.8 percent arachidonic acid. This was in agreement with Klein and Martin (1959) who also reported differences in liver and serum cholesterol esterified fatty acids. These authors speculated that the differences might be due to varying turnover rates for the different esters.

Mukherjee et al. (1957) reported that oleic acid was absent from serum cholesterol esters while Klein and Janssen (1959) published that serum cholesterol esters contained 59 percent oleic acid. These differences might have been due to analytical technique, or, more likely to the state of the rat when samples were taken (Swell et al., 1960c). Transesterification reactions between phospholipids and cholesterol esters may occur (Glomset et al., 1962). Brot et al. (1964) and Aftergood et al.

(1956) are in agreement that approximately 75 percent of the liver cholesterol exists in the free form.

Reiser et al. (1960) reported that triglycerides from the plasma, liver and depot fat of the miniature pig were not related as measured by their fatty acid composition. Furthermore, only depot fat triglycerides responded to differences in the lipid composition of the diet. The triglyceride composition of polyunsaturated fatty acids in perirenal fat, plasma and liver on low fat, 20 percent myristyl oleyl linoleate and 20 percent cottonseed oil were as follows:

	Perirenal		Plasma	Liver	
	Dienoic	Trienoic	Dienoic	Dienoic	Trienoic
Low fat	4.0	0.7	7.8	3.2	0.6
20% M-O-L	3.8	0.9	8.2	2.3	0.0
20% CS^0	33.5	1.1	9.7	1.6	0.0

Since the level of dienoic fatty acid was twice as high in blood plasma as in other tissues, Reiser et al. (1960) concluded that the plasma triglycerides probably did not originate in the liver. In the presence of readily available carbohydrates, saturated fatty acids were utilized for energy to a greater degree than unsaturated fatty acids and therefore, were stored in depot fats to a lesser degree. Okey et al. (1962) were in agreement that plasma and liver lipids were less subject to changes than the adipose tissue when various diets were fed. They also reported that lauric and myristic acid tended to accumulate preferentially in the adipose tissue.

Conversely, Swell et al. (1962) reported that, in rabbits, the nature of the dietary fat incorporated into a high cholesterol diet influenced the serum and tissue cholesterol ester and triglyceride fatty acid composition.

In an analysis of the phospholipid fraction from rat liver, Getz et al. (1961) found 62 percent linoleic acid, 15.6 percent oleic acid and 8.9 percent palmitic acid. This work was in agreement with Dittmer and Hanahan (1959) and Macfarlane et al. (1960).

When Patil and Magar (1960) fed vegetable fats to rats, the liver levelsof dienoic acid in the cholesterol ester and phospholipid fractions wereelevated. The phospholipid fraction was found to have a higher content of tetraenoic, pentaenoic and hexaenoic acids than the cholesterol ester and triglyceride fractions.

Nestel and Steinberg (1963) reported rat liver glyceride and phospholipids to have the following composition; myristic acid, 1 percent each, palmitic acid 23 and 14 percent, palmitoleic acid, 3 and 7 percent, stearic acid 4 and 15 percent, oleic acid 36 and 27 percent, linoleic acid 32 percent each and arachidonic acid 1 and 5 percent, respectively. Except for differences in the stearic acid levels, these figures are in agreement with Getz et al. (1961).

In the analysis of liver free fatty acid and diglyceride fractions, Getz et al. (1961) found high levels of palmitic acid, 30.6 and 23.9 percent, stearic acid, 10.5 and 6.6 percent, oleic acid, 25.0 and 28.8 percent and linoleic acid, 19.5 and 34.1 percent, respectively. These levels for free fatty acids are in agreement, generally, with Nestel and Steinberg (1963) and Rose et al. (1964).

Nichaman et al. (1963) reported that the cholesterol esters of human blood serum contained 15.5 percent palmitic acid, 19.6 percent stearic acid, 52.7 percent linoleic acid and 7 percent arachidonic acid.

Mukherjee et al. (1957) examined the influence of fat free rations and stock rations on the triglyceride, cholesterol ester and phospholipid fractions of rat serum. They reported that of these fractions, 49 percent was in the form of phospholipids, 33 percent as cholesterol esters and 18 percent as triglycerides. The fatty acid composition was as follows:

Fatty acid	Saturated	18:1	18:2	18:3	20:4*
Stock ration					
Triglyceride	76.5	0.0	18.0	1.0	1.0
Cholesterol ester	55.8	0.0	25.0	1.5	9.8
Phospholipid	71.7	0.0	10.4	0.6	13.2
Fat free ration					
Triglyceride	21.0	72.0	1.6	4.3	1.1
Cholesterol ester	30.1	53.0	9.0	1.3	3.6
Phospholipid	47.3	41.2	3.5	6.7	1.3

*Fatty acid abbreviation system suggested by Dole et al. (1959)

Mead (1957) suggested that the synthesis of the monoenoic fatty acid might be accelerated in cases of fat deficiency to maintain a certain degree of total unsaturation and physical properties of the tissue lipids which under normal conditions were provided by dietary essential fatty acids and their metabolites. Mohrhauer and Holman (1963) were in agreement, finding that the oleic content of the total liver lipids was increased to 40 percent when a fat free diet was fed.

Okey and Lyman (1957) found that serum phospholipids showed some tendency to vary in the same direction as serum cholesterol. They suggested that this might be taken to indicate a connection between the level of circulating cholesterol and that of phospholipids and would be in line with the evidence that a large part of the circulating cholesterol is in combination in giant molecules containing protein, phospholipids and free fatty acids.

The influences of the dietary lipid composition on serum cholesterol levels have been intensely investigated. Kinsell et al. (1952) reported the first findings that vegetable fats tended to lower serum cholesterol levels in man. Since that early "letter to the editor", the hypothesis that vegetable fats do lower serum cholesterol levels has been supported by Ahrens et al. (1954), Hardinge and Stare (1954), Bronte-Stewart et al. (1956), Ahrens et al. (1957a), Ahrens et al. (1957b), Brozek et al (1957), Horlick and Craig (1957), Brown and Page (1958), Jolliffe et al. (1959).

Keys et al. (1956) disagreed to some extent, explaining that the level of fat in the diet had greater effect on lowering serum cholesterol levels than did the degree of unsaturation. Pollack (1959) reported that highly unsaturated, low fat diets resulted in a higher incidence of cardiovascular disease in Japanese when compared to a highly saturated, high fat diet in Thailand.

James and Lovelock (1958) reported that the assumed relationship between low essential fatty acid intake and coronary artery disease rests upon two proved claims; firstly, an inverse relationship between linoleic acid intake and blood cholesterol levels in man, and secondly, an asso-

ciation between low essential fatty acid intake and atheroma in animals. However, they concluded that at this stage of development in the study of coronary artery disease, it can be stated unequivocally that there is no adequate evidence to show that a deficiency of essential fatty acids is a factor in its causation.

Mohrhauer and Holman (1963b) reported that on fat free diets, the subcutaneous fats contained 29.3 percent palmitic acid, 18.5 percent palmitoleic acid and 47.7 percent oleic acid. These results are in agreement with the findings of Mead (1957) and Reiser et al. (1960).

Webb et al. (1961) and James et al. (1961) reported that the fecal fats of humans did not closely resemble the dietary fats. Odd isomers of oleic acid and 10 hydroxy-stearic acid were present in the feces. Tests proved that stearic acid is the precursor of both acids. The major fatty acids of the feces in free and bound forms were myristic acid, 8.9 and 4.4, palmitic acid, 55.2 and 35.3, stearic acid, 12.9 and 31.8, oleic acid (including isomer) 10.3 and 17.2, and linoleic acid, 1.3 and 2.8 percent, respectively.

Hoet et al. (1963) reported that the odd isomers of various fatty acids in the feces of rats are products of the intestinal bacteria. On a fat free diet, rat feces contained 27.4 mg fatty acids per gram of dry feces. The percent fatty acid composition was: lauric, 1.0; myristic, 3.4; palmitic, 38.9; palmitoleic, 2.6; stearic, 24.9; oleic, 17.9; and linoleic acid, 11.2. The fat in this case is derived from endogenous sources such as bile secretions, desquamated mucosa cells, mucosal secretions and from bacteria. When 21 percent corn oil was added to the diet,

the feces contained 44.3 percent palmitic, 23.8 percent stearic, 25 percent oleic and 8.5 percent linoleic acid. The fat excretion increased only slightly with increasing fat ingestion.

Spritz and Ahrens (1963) reported that the neutral sterols and bile acids of feces represent more than 99 percent of the excretion products of cholesterol under normal conditions. The identification of the sterols has been hindered by multiple variations in chemical structure produced by intestinal bacteria and the complexity of substances with which sterol extracts are contaminated. The sterols were primarily cholesterol and coprostanol which accounts for 90 percent of the sterols and bile acid in feces.

Schoenheimer and Sperry (1934) found that cholesterol was excreted as coprosterol and dihydrocholesterol. Since only 11 percent of the total sterols was unsaturated, the cholesterol content of feces was very low. Siperstein and Chaikoff (1952) agreed that only a very small fraction of body cholesterol is excreted as non-saponifiable compounds, i.e. cholesterol, coprosterol or dihydrocholesterol. The majority of C^{14} from a dietary cholesterol source was found as saponifiable compounds, i.e. bile acids, in feces.

In studies on the activity of different segments of the rat intestine during absorption of iodine-labeled olive oil, Benson et al. (1956) found maximum activity in the distal jejunum. Based on the assumption that the segment with the highest activity represents the site of maximal absorption, they concluded that the distal jejunum was the primary site of fat absorption.

Frazer (1962) stated that the chain length of a fatty acid may affect its digestibility. Long-chain fatty acids such as palmitic and stearic acid, are poorly absorbed as compared with shorter-chain compounds. The addition of a liquid oil vehicle to the long-chain saturated fatty acids causes a marked improvement in absorption. Deuel (1955) reported the relationship between melting point and digestibility as follows:

	Melting point °C	Digestibility coefficient in rat
Myristic acid	53	82
Palmitic acid	63	36
Stearic acid	69	16
Oleic acid	14	95
Elaidic acid	51	96

Shoreland et al. (1957) reported that ruminants had no special difficulty in the absorption of trans acids. On the other hand Deuel (1955) indicated that the higher melting trans acids behaved more similarly to the more suturated fatty acids in guinea pigs.

According to Frazer (1962) there is probably selective absorption or more specifically, selective rejection of certain fatty acids even though they might have low melting points. When erucic acid (melting point 33.5°C) was fed, the proportion of erucic acid in the feces fat was significantly higher than the proportion in the fat fed. Also, as shown by James et al. (1961) hydroxy-fatty acids are not absorbed to any degree.

Frazer and Sammons (1945), Desnuelle et al. (1948), Mattson et al. (1952) and Mattson and Beck (1956) have shown that pancreatic lipase did

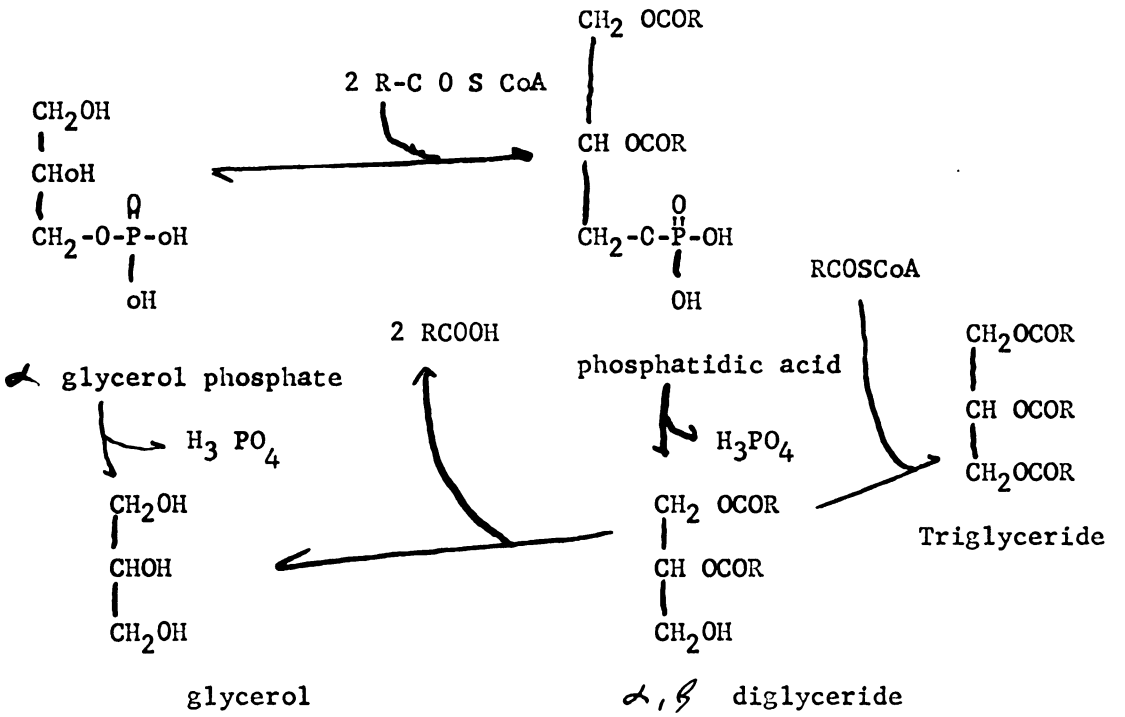
not rapidly and completely hydrolyze dietary glyceride to fatty acids and glycerol. The end products were di- and mono-glycerides, fatty acids and relatively little free glycerol. Desnuelle et al. (1948) and Mattson and Beck (1952) have shown that pancreatic lipase has a selective action on fatty acids in a triglyceride, the fatty acids in the one (α) and three (α') positions being preferentially liberated. Frazer and Sammons (1945) listed four sources of lipase: gastric, pancreatic and intestinal secretion and lipoclastic bacteria. Pancreatic lipase was the most active.

Frazer (1960) described the differences between fat emulsion in the intestinal lumen and fat particles is the chyle:

	Intestinal lumen	Chyle
Composition	Triglycerides	Triglycerides
	Diglycerides	containing long-
	Monoglycerides	chain fatty acids
	Short-chain fatty acids	Phosphatidyl choline
	Long-chain fatty acids	Cholesterol esters
Surface film	Lower glyceride-fatty acid-bile salt complex	Lipoprotein

He concluded that triglycerides were reformed and that long-chain fatty acids, with more than 10 carbons in the chain, are selected for this esterification. The shorter-chain fatty acids did not re-enter glycerides; nor was liberated glycerol re-utilized in the intestinal cell for glyceride synthesis. Fatty acids were also taken up into phosphatides, especially lecithin, and into cholesterol esters. For the latter, there was a preferential selection of more unsaturated fatty acids. Two pathways for glyceride syntheses have been demonstrated in the intestinal cell. The

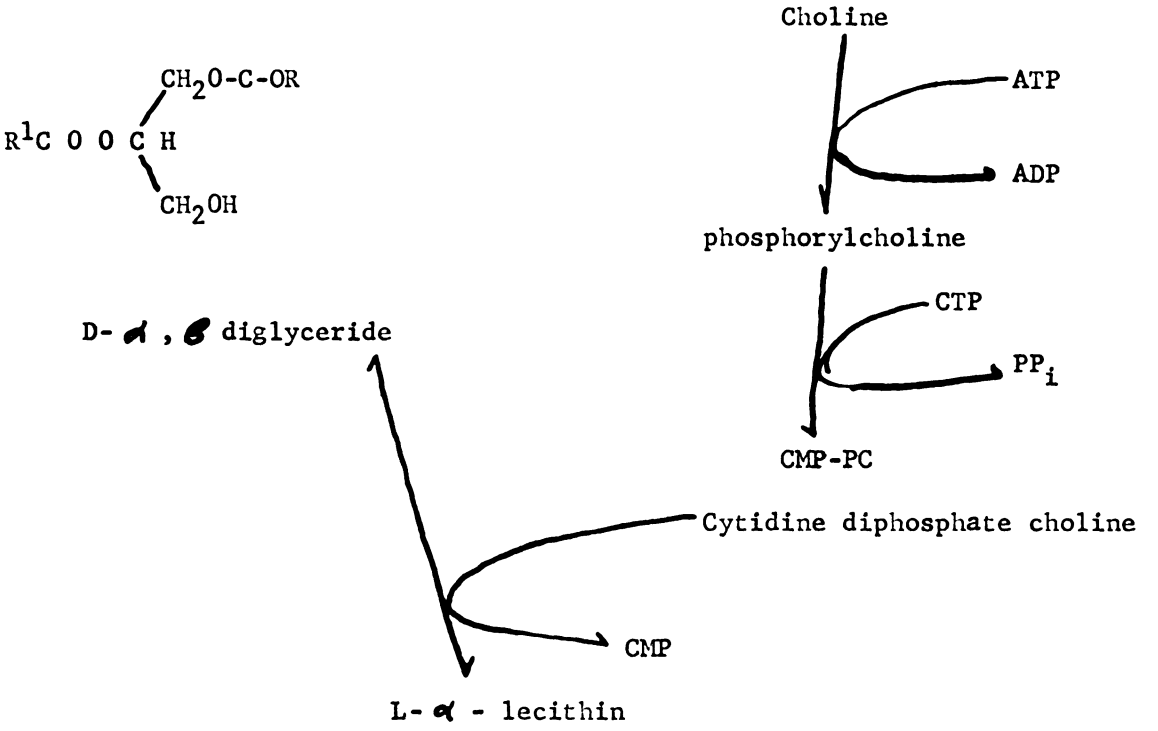
pathway described by Kennedy (1957) involves phosphatidic acid as an intermediate. According to Clark and Hübscher (1960), this pathway operates in the small intestine as follows:



Buell and Reiser (1959) proved that the free glycerol liberated from lipase hydrolysis of the di-or mono-glycerides could not be re-utilized for triglyceride or glycerol phosphate synthesis in the intestinal mucosa. They stated, however, that there was a possibility that free glycerol can be used in the liver for α glycerol phosphate synthesis.

A pathway described by Clark and Hübsher (1961) allowed direct incorporation of fatty acids into lower glycerides. Therefore, according to Frazer (1962), the composition of the dietary glyceride will affect the glycerides resynthesized in the intestinal mucosa.

Kennedy and Weiss (1955), (1956) described a pathway allowing for the incorporation of D-α,β diglycerides into the phospholipid, lecithin, as outlined below:



According to Kennedy and Weiss (1956) a similar pathway involving the active intermediate cytidine diphosphate ethanolamine is responsible for the formation of phosphatidyl ethanolamine.

In studies on the fatty acid composition of lecithin from various sources, Tattrie (1959), Hanahan et al. (1960) and Marinetti et al. (1960) found that there was a specific location of the saturated and unsaturated fatty acids. The saturated fatty acids were located at the α-ester position and unsaturated fatty acids were found preferentially located at the β-ester position of egg lecithin, bovine plasma lecithin, and rat liver lecithin.

Hilditch and Stainsby (1935) stated that lard triglycerides contained the saturated fatty acid, palmitic acid, almost exclusively at the β-position. Young (1961) reported that lard contains unsaturated fatty acids in the β-position in only 28 percent of the total triglycerides. Mattson et al. (1964) indicated that palmitic acid is found predominantly at the β-ester position in the triglycerides of the domestic pig. Myristic acid also tended to concentrate at the β-position. The anatomical position from which adipose tissue was taken did not influence the distribution of fatty acids on the triglyceride molecule.

Mattson et al. (1964) also reported that the composition of the dietary fat had no effect on the adipose triglycerides of pigs as shown below:

Fatty acid	14:0	16:0	16:1	18:0	18:1	18:2	18:3
Total F.A. Mole %[x]	1	28	3	15	3	42	2
β-ester fatty acid	5	72	4	4	12	3	0
% F.A. in β position	167	86	44	9	10	11	0

The fatty acid composition of triglycerides from pigs on a safflower oil diet was as follows:

Fatty acid	14:0	16:0	16:1	18:0	18:1	18:2	18:3
Total F.A. mole %[x]	1	3	1	8	20	54	3
β-ester F.A.	2	36	2	3	12	42	2
% F.A. in β-position	67	92	67	12	20	26	22

x The analytical error was seven percent.

An analysis of soybean oil by Mattson and Volpenheim (1963) revealed that the β-ester of triglycerides was primarily occupied by unsaturated fatty acids. The composition was given as follows:

Fatty acid	16:0	18:0	18:1	18:2	18:3
Total F.A. mole %	12	4	25	51	8
β-ester F.A.	1	0	22	69	8
% F.A. on β ester	3	0	29	45	33

EXPERIMENTAL PROCEDURE

Source of Animals

The Carworth strain of albino rats used in this study were obtained from the Michigan State University Animal Husbandry Department rat colony. Males weighing 500 g ± 150 g at 18 to 24 months of age were selected since this physiological age approximates a human's middle age. (Kleiber, 1961).

The rats were divided into three groups of four animals on the basis that the weights be distributed through all groups in a manner as uniform as possible.

Three rations were formulated to supply 76 Calories (Wagner, 1962) daily to each rat. The basic diet consisted of low fat ingredients as suggested by Wooley and Sebrell (1945). The basic ration was found to provide 4.1 C/g by a Parr Oxygen Bomb Calorimeter gross energy determination (Appendix S).

The lipid portions of the rations were isolated from soybean oil and lard. In order to obtain a vegetable lipid containing 33 percent polyunsaturated fatty acids, refined soybean oil was fractionally crystallized from acetone at -11°C. As shown in Table 1, the crystallization involving 10 percent soybean oil in acetone (W/W) provided the required lipid.

A like procedure was attempted with open kettle rendered lard obtained from the Michigan State University Meat Laboratory. A fraction containing 24 percent polyunsaturated fatty acids was isolated from

Table 1. Fatty Acid Composition of Fractions Crystallized from Acetone[x]

Percent soybean oil in acetone W/W	10	15	20	25
Myristic acid	1.1[xx]	3.2	1.7	0.3
Palmitic acid	21.0	26.5	24.1	21.0
Stearic acid	15.1	9.8	11.6	11.2
Oleic acid	27.3	19.3	19.3	22.6
Linoleic acid	29.5	38.6	37.1	37.3
Linolenic acid	5.9	2.5	6.2	7.5
Percent polyunsaturated F.A.	35.4	41.1	43.3	44.8

[x] Uncorrected area percent by gas chromatography.
[xx]Average of three determinations of methyl esters prepared from the fraction.

acetone at -26°C. Since this lipid fraction did not meet the specifications previously stated, a barrow was fed a diet containing 20 percent corn oil for 17 days. The panniculus adiposus was finely chopped and rendered under nitrogen gas in an autoclave at 121°C for two hours. The lipid layer was removed and centrifuged at 2100 rpm for 15 minutes. The lard obtained by this procedure contained 34 percent polyunsaturated fatty acids. The fatty acid composition was shown to be myristic, 0.7; palmitic, 18.2; palmitoleic, 9.7; stearic, 7.2; oleic, 30.1; linoleic, 26.4; and linolenic acid, 7.8 percent. Differential cooling curves as described by Jacobson et al. (1961) were used to prove the resultant triglycerides were not composed entirely of saturated or unsaturated fatty acids. The presence of extremely small amounts of diglycerides in both lipid samples was revealed by thin-layer chromatography (Mangold, 1961).

The first group of rats was maintained on the basic fat free ration (diet A) for a period of two weeks. The second and third groups were maintained at an iso-caloric level with the first group. Forty percent of the calories in each case was supplied by either the lard lipid fraction (diet B) or the soybean oil lipid fraction (diet C). The rats were caged separately, placed randomly on the racks and fed daily for a two week period.

Feces samples were collected by placing a paper towel under each cage for a 24 hour period. The samples were weighed and stored under nitrogen gas at -30°C until the analyses were performed.

At the end of the two week dietary regimes, the rats were decapitated 24 hours after feeding and held over a funnel in order to collect blood samples. Massaging increased the amount of blood collected from each rat. The samples were centrifuged at 2800 rpm and 5°C for 15 minutes. The blood serum was decanted and stored under nitrogen gas at -30°C. The abdominal cavities were opened, and liver, aorta, and perirenal fat samples were taken. The livers were blotted on paper towels and weighed. The extraneous fatty material was removed from the aortas. Subcutaneous fat samples were taken from the area of the last lumbar vertebra. These samples were stored under nitrogen at -30°C.

Extraction of Lipids

The lipids were extracted from the liver, blood, feces and pooled aorta samples by a modification of the method suggested and recommended by Ostrander and Dugan (1962). Each sample was placed in a VerTis "45"

flask with 130 ml absolute methanol and macerated with a VerTis "45" homogenizer for five minutes at medium speed. The sample was transferred to a Waring Blender jar with 65 ml chloroform and blended for five minutes. The VerTis "45" flask was rinsed with 65 ml chloroform which was then added to the Waring Blender jar and blended for 30 seconds. In order to precipitate the protein in the sample, 65 ml distilled water containing one to one and a half g zinc acetate was added and blended for 10 seconds. The sample was filtered by suction on a Buchner funnel using Whatman No. 1 filter paper. Nitrogen gas was directed over the sample during this process. The residue and filter paper were returned to the blender jar along with one half of a filter paper used to wipe the funnel. To this residue 100 ml chloroform was added and the sample was blended two and a half minutes and then filtered as before. The blender jar and residue were rinsed with 50 ml chloroform. The total filtrate was transferred to a separatory funnel and the heavy chloroform layer was removed for lipid analysis. Remaining lipids in the aqueous layer were extracted with 100 ml chloroform. The solvent was removed from the chloroform layer under reduced pressure by means of a rotating flash evaporator. The lipid concentrations of the liver, feces and blood samples were calculated on a gram or ml basis. The dry samples were stored under nitrogen at -30°C. The solvent system for the blood serum and pooled aorta samples was reduced by one-half.

The phospholipids were separated from the neutral lipids by a modification of a method reported by Bates (1958), Reiser et al. (1960) and Choudhury and Arnold (1960). Each lipid sample was dissolved in 50 ml

chloroform and mixed with 10 g silicic acid and stirred with a magnetic stirrer for 10 minutes. This operation was performed under a direct stream of nitrogen gas. The chloroform was filtered off by suction through a sintered glass funnel and the silicic acid was washed with four 50 ml portions of chloroform. The solvent was removed by reduced pressure and the weight of the neutral lipids was obtained.

The silicic acid was then washed with four 50 ml portions of methanol to remove the phospholipids. The weights of the phospholipid fraction were recorded following the removal of the methanol under reduced pressure at 70°C.

Thin-Layer Chromatography

Following unsuccessful attempts to achieve the complete separation of neutral lipid classes on Florosil columns (Carroll, 1961) thin-layer chromatography was employed. Camag equipment consisting of 20 by 20 cm glass plates, spotting template, applicator and chromatographic developing tank was used. Anasil B, a thin-layer chromatographic silica gel adsorbant containing a calcium sulfate binder, was slurried with distilled water in a ratio of 1:2 (W/V) and applied to the glass plates in an uniform layer about 250 microns in thickness. The plates were allowed to dry at room temperature for 15 minutes and then were activated by heating in an oven at 105°C for one hour. The activated plates were stored in a desiccator until they were required for analytical procedures. Capillary tubes were used to apply the samples to the chromatoplates. The plates were developed in a equilibrated chromatographic tank utilizing

100 ml solvent consisting of 80 ml hexane, 20 ml diethyl ether and one ml concentrated acetic acid which was added to prevent excessive tailing. After the solvent traveled 16 cm up the plate, the chromatoplates were allowed to dry at room temperature. Visualization of the developed plates was accomplished by spraying with five percent phosphomolybdic acid in ethanol or 50 percent sulfuric acid in 50 percent ethanol to which two percent ferric chloride was added. The plates were then charred in an oven at 105°C and the spots were traced on acetate paper. Cholesterol, cholesterol acetate, oleic acid and triolein were used as standards for cholesterol, cholesterol esters, free fatty acids and triglyceride classes.

Preparation of Methyl Esters

Methyl esters of the fatty acids were made utilizing a method developed by McGinnis and Dugan (1964) and Dugan and McGinnis (1964). One g fat samples were suspended in 20 ml diethyl ether and homogenized with a VerTis "45" homogenizer. The homogenate was transferred to a 125 ml Erlenmeyer flask mounted in an ice bath on a magnetic stirrer. Under a constant stream of nitrogen gas, two and one half ml concentrated sulfuric acid were added dropwise to the flask. The flask was stoppered and stirred for 10 minutes reaction time, after which, a drop of alcoholic phenolphthalein was added and the mixture was titrated with 3.6 N methanolic potassium hydroxide. The neutral mixture was transferred to a separatory funnel, the reaction flask rinsed with diethyl ether, and the solvent was washed with cold, distilled water. The aqueous layer was

discarded and the diethyl ether layer was dried over anhydrous sodium sulfate. The sample was filtered through Whatman No. 1 filter paper and the solvent was removed under a stream of nitrogen gas. The resulting methyl esters of fatty acids were injected into the gas chromatograph for qualitative and quantitative analysis.

The neutral lipids from the liver, feces, blood serum and pooled aorta samples were separated into classes on thin-layer chromatoplates. Approximately seven mg samples were spotted across the plate and a reference mixture was spotted at the boundary. Following the development of the plate, a cover glass plate was taped over the absorbant surface excluding the area occupied by the reference material and one neutral lipid unknown. This area was sprayed with 50 percent sulfuric acid in 50 percent ethanol plus two percent ferric chloride. The plates were charred and the various lipid classes were identified by relative Rf values and the red color developed by spots containing cholesterol. The lipid classes were scraped into 125 ml Erlenmeyer flasks and suspended in 20 ml diethyl ether. The esterification procedure was followed as in the fat analysis. The phospholipid fractions were dissolved in 20 ml diethyl ether and esterified in the same manner. In order to evaluate the accuracy of the procedure, 4 mg of palmityl oleyl stearin, obtained through the courtesy of R. J. Vander Wal, Armour and Company Research Laboratories, was analyzed by this procedure. This specific triglyceride yielded 38 percent palmitic acid, 30 percent each of oleic and stearic acids and two percent myristic acid by gas chromatographic analysis.

Gas Chromatography

A Barber-Colman Model 20 gas chromatograph equipped with a radium ionization detector and a Barber-Colman recorder was used. For most of the analyses, the following adjustments were maintained: argon gas pressure, 30 lb.; argon gas flow rate, 151 ml per minute; injector port and detector temperatures, 240°C; column temperature, 170°C; cell voltage, 1250V; sensitivity, 1 x 10^{-7} amps full scale; split flow, 200 ml per minute; and column, 6 ft by 1/4 in. copper tubing with 12 percent ethylene glycol succinate on 60/70 mesh Anakron A or 20 percent diethylene glycol succinate with two percent phosphoric acid on 60/80 mesh Chromasorb W. The columns were packed using an electric vibrator and then coiled to a diameter of five inches. The columns were preconditioned at 200°C with an argon flow rate of 150 ml per minute for at least 24 hours.

The detection system was checked for quantitative accuracy by injecting aliquots of an equal mixture of the methyl esters of stearic acid, oleic acid, linoleic acid and linolenic acid, and demonstrating that under the conditions used, the peak area for each ester averaged 25 percent of the total. The results of quantitative analysis of fatty acid composition reported were taken directly from areas under the curve without corrections for possible variations in the response of the detector to different molecular species. The analysis also did not include long chain methyl esters present with retention times greater than methyl arachidonate. The retention times of various methyl esters (99+ percent pure) were compared to the retention times of the unknown methyl esters for qualitative analyses.

When standards were not available, peaks were tentatively identified by semilogarithmic plots of retention volumes against carbon number.

Cholesterol Determinations

The total and free cholesterol content of blood serum were measured by the method published by Ferro and Ham (1960). A modification of this method was used for liver cholesterol measurements. The total liver lipid extract was dissolved in 100 ml diethyl ether and duplicate 10 ml samples were taken. The solvent was then removed from each sample under reduced pressure. The residue was dissolved in 10 ml isopropyl alcohol and one half ml was taken for analysis. Four and one half ml isopropyl alcohol were added to the sample, which was then shaken vigorously and permitted to stand for 10 minutes. The sample was then centrifuged for 10 minutes at 3000 rpm at 5°C.

For total cholesterol measurements, one ml of the supernatant was placed in a 16 x 100 mm test tube. One half ml alcoholic potassium hydroxide (5 percent KOH in 95 percent ethanol) was mixed with the supernatant sample which was then placed in a water bath at 37°C for 30 minutes. The tubes were removed from the water bath and one drop of phenolphthalein (one percent in ethanol) was added. The mixture was titrated with 10 percent acetic acid until the color disappeared, and then one additional drop was added.

Free cholesterol measurements were made by placing two ml of the isopropyl alcohol supernatant in 16 x 100 mm test tubes. To both the neutralized total and free cholesterol samples, one ml digitonin solution

(one g digitonin in 50 ml 95 percent ethanol and diluted to 100 ml with distilled water) and one drop of 30 percent aluminum chloride were added. The aluminum chloride acted as a gathering agent for the digotonide precipitate. The samples were mixed by swirling and allowed to stand for 30 minutes. The tubes were centrifuged at 3000 rpm for 10 minutes and the supernatant was removed by suction. The packed precipitate was washed thoroughly with 3 ml acetone, centrifuged, and the supernatant was removed by suction. The tubes were inverted and drained over a paper towel to insure that no precipitate was lost. The precipitate was broken by adding 0.2 ml distilled water and shaking. A color development mixture was prepared by mixing three volumes reagent grade acetic anhydride with two volumes reagent grade acetic acid. To 10 parts acid-anhydride stock solution, 1 part reagent grade concentrated sulfuric acid was added and the mixture was cooled to room temperature. Six ml of the color development mixture were added to the digitonide precipitate. The solution was transferred to a cuvette and the peak absorption at 640 mμ against a water blank was observed within 90 $\pm$ 30 seconds. Matched cuvettes were used in a Baush and Lomb Spectronic 20 colorimeter. Liver cholesterol standards were prepared by adding 100 mg free cholesterol to 100 ml isopropyl alcohol. Standards were then taken with 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.8 and 1.0 mg cholesterol. The standards were prepared for absorption readings by the procedure described for free cholesterol.

The optical densities of the unknowns were read against a standard curve. The values obtained for free cholesterol were divided by two. All readings were then based on one percent of the total lipid extract,

and this factor was used in estimating the total and free cholesterol content of the samples. The values were then converted to a mg cholesterol per g wet tissue basis.

Statistical Analysis

The statistical analyses were based on the methods of Kempthorne (1952), Duncan (1955) and Snedecor (1956). Analysis of variance and multiple range tests were employed.

RESULTS AND DISCUSSION

On the fat free (diet A), lard (diet B) and soybean oil (diet C) diets, one rat on each died of undetermined causes before the test period was completed. The deceased rats lost little weight and showed no external signs of sickness. The caloric consumption per rat is given in Table 2.

Table 2. Dietary Record

Diet	Rat No.	Days on test	Total Calories	Calories /day	Final weight (g)	Weight change (g-)
A	1	14	1064	76	499	-51
A	2	14	1064	76	501	-60
A	3	14	1064	76	535	-77
A	4[1]	12	891	74.3	457	-45
B	1	14	1064	76	515	-41
B	2	14	1064	76	565	-44
B	3[2]	4	278	69.5	650	-7
B	4	14	1064	76	479	-28
C	1[3]	12	760	63.3	375	+11
C	2	14	1064	76	515	-34
C	3	14	1064	76	516	-46
C	4	14	1064	76	628	-48

[1]On the three days prior to death, caloric consumption was 57.5, 49.3 and 24.6 C/day.
[2]On the day prior to death, caloric consumption was 51.5 Calories.
[3]The rat stopped eating 2 days prior to death.

Due to the deaths, statistical significance at the P = .05 level was not observed in many expected cases since the "F" ratio was necessarily extremely high (6.94). For that reason, data approaching significance and data indicating possible trends are reported herein. Furthermore, since the investigation was primarily designed to study the effects of

diet on the di-, tri-, and tetraenoic (polyunsaturated) fatty acid composition of the tissues, the analyses generally considered only the percentage of polyunsaturated fatty acids in each lipid component.

Contrary to the report of Hoet et al. (1963) increases in the fat content of the diet significantly increased the level of feces fat. However, Hoet et al. (1963) compared rats on fat free rations with rats on 21 percent corn oil diets. In the present study, the rations containing lipids furnishing 40 percent of the total calories produced significantly higher feces lipid levels as shown in Tables 3 and 4.

Table 3. Analysis of variance of lipid content[1] of rat feces.

Source	df	M. S.	F
Replicates	2	10.05	2.49
Diets	2	86.69	21.46**
Replicates x diets	4	4.04	

**P < .01
[1]Appendix A.

Table 4. Multiple range test of rat feces lipid content.

Number of pairs	(2)	(3)
Shortest significant range	5.58	5.69
Diet	A	B	C
Percent lipid	3.12 ± 0.66	12.00 ± 3.28	12.80 ± 2.63

Note: Any two means not underscored by the same line are significantly different.

Perhaps the physiological capability of the lipid absorption mechanism has been exceeded at this high level of dietary fat. Furthermore, age effects may have some influence on lipid absorption.

Table 5 presents the fatty acid composition of the various neutral lipid fractions isolated from the feces. Cholesterol esters were not detected in the neutral lipids. Statistical examination of the effects of diet and fraction on the fatty acid composition revealed no significant differences.

There appear to be major differences in the rate at which various fatty acids were absorbed. Diets containing 33 percent polyunsaturated fatty acids produced feces containing five percent polyunsaturates. One percent myristic acid in the diet yielded 45 percent myristic acid in the feces. This is, perhaps, explained by Hoet et al. (1963) who reported that the greatest percentage of feces lipids were of endogenous origin. Frazer (1962) offers a further clue in that some fatty acids are selectively rejected or absorbed in the digestive process. In the present case, the author would postulate that the polyunsaturated fatty acids were preferentially absorbed at the expense of palmitic and stearic acids. There was apparently a trend towards greater absorption of polyunsaturated fatty acids from diet B. The diets, A, B and C, produced feces triglycerides yielding 5.8, 3.9, and 6.8 percent polyunsaturated fatty acids, respectively. In the diglyceride fraction, the results were 6.4, 5.2 and 10.2 percent, respectively, and in the monoglyceride-free fatty acid fraction the percentages were 2.1, 1.7 and 33.3, respectively. The latter figure produced by diet C was postulated to be due to the structure of the dietary

Table 5. Fatty acid composition[1] of rat feces neutral lipids

Fatty acid	Triglyceride			Diglyceride			Monoglyceride & free fatty acid		
	Diet A	Diet B	Diet C	Diet A	Diet B	Diet C	Diet A	Diet B	Diet C
12:0	0.4± .7	T	0	T	0.6±1.0	0	0	0	0
14:0	64.3±13.8	46.3±5.9	49.6±6.0	75.3±5.1	65.1±0.9	65.8±11.2	73.9±13.8	75.8±13.2	52.3±33.2
16:0	12.3± 2.2	15.7±2.6	17.9±2.7	8.0±3.0	12.5±2.1	13.1± 6.5	12.1±10.4	10.9± 3.0	6.5± 2.4
16:1	5.7± 1.0	5.8±1.2	3.9±2.6	3.9±1.5	5.4±0.9	2.6± 1.1	4.5± .6	2.5± 0.8	2.7± 1.6
18:0	0.8± 2.1	5.9±8.7	1.3±1.6	0.7±0.8	1.5±1.8	1.1± .4	T	0.8± 0.7	1.1± .8
18:1	10.5± 9.2	18.7±6.9	19.5±2.8	4.1±3.0	8.7±2.1	7.1± 2.3	5.5± 3.9	6.0± 5.3	4.0± 2.1
18:2	3.9± 2.1	5.8±4.7	4.4±4.5	2.6±1.5	2.6±1.7	2.9±2.7	1.5± 1.4	3.3± 3.1	1.9± 1.2
18:3	T	T	0.4±0.5	T	0.3±0.4	T	T	0.4± 0.7	T
20:4	1.3± 1.7	0.8±1.4	2.0±3.3	1.7±2.8	1.6±2.7	3.2± 5.5	0.6± 1.1	0	22.9±25.9
A[2]	T	T	0	T	0	2.3± 3.9	0	0	0
B[2]	T	T	0	1.9±3.2	1.7±1.1	0	0	0	0
C[2]	0.8± 1.4	0	0	T	0	0	0	0	T
D[2]	0	0	0	0	0	1.9± 3.2	0	0	0

[1]Appendices B, C and D
[2]The identification of these fatty acids by log retention volume versus carbon number were tentatively identified as 20:1, 20:2, 20:3 and 22:1, respectively.

lipid. Pancreatic lipase preferentially attacks the α and α' ester linkages leaving the β position intact (Desnuelle et al., 1948; Mattson and Beck, 1952). Since the unsaturated fatty acids were preferentially esterified at the β-position (Mattson and Volpenheim, 1963), the resultant monoglyceride would be expected to contain large amounts of polyunsaturated fatty acids. The same reasoning would explain the low (1.7 percent) polyunsaturated fatty acid content of the monoglyceride-free fatty acid fraction of the feces produced by diet B. Here, the β-ester is occupied primarily by saturated fatty acids (Mattson et al., 1964).

The dietary effect on the neutral lipid content of the total feces lipids approaches significance at the $P \leq .05$ level as indicated in Table 6.

Table 6. Analysis of variance of the neutral lipid content[1] of feces lipids.

Source	df	MS	F
Replicates	2	2.62	0.21
Diet	2	74.02	5.98
Replicates x diets	4	12.38	

[1]Appendix A.

The mean neutral lipid content of the feces lipids produced by diets A, B and C were 31.77 ± 0.37, 24.50 ± 3.45 and 34.00 ± 2.55 percent, respectively. The level of phospholipids in the total feces lipids tends to be elevated in the feces of rats fed diet B. Oxidation of the feces phospholipids prevented a fatty acid analysis.

Analysis and statistical examination of the fatty acids in the subcutaneous and perirenal fats indicated that a significant difference was found only in the content of polyunsaturated fatty acids in each kind.

Table 7. Analysis of variance of polyunsaturated fatty acid content[1] of subcutaneous and perirenal fats.

Source	df	MS	F
Replicates	3	29.05	0.77
Diet	2	37.07	0.99
Replicate x diet	6	37.55	
Location	1	71.22	5.56*
Diet x location	2	15.71	1.23
Replicate x diet x location	9	12.80	

*$P < .05$
[1]Appendices E and F

The polyunsaturated fatty acid content of the subcutaneous fat (36.19 $\pm$6.59 percent) was significantly less than that of the perirenal fat (39.59 $\pm$2.37 percent). The saturated fatty acid content was 22.52$\pm$7.75 and 20.98 $\pm$2.11 percent for the subcutaneous and perirenal fats, respectively. Overall, the oleic acid content was 34.37$\pm$0.42 percent and palmitoleic acid, 5.88$\pm$2.79 percent.

Examination of Table 8 suggests trends in relation to the dietary fat. The rats on diet A had the lowest content of saturated and highest levels of mono- and polyunsaturated fatty acids in their body fats. This may indicate that insufficient amounts of saturated fatty acids were syn-

thesized from non-fat dietary sources and that the individual was forced to mobilize saturated fatty acids from the fat deposits in order to meet physiological requirements. In the case of rats on diets B and C, it might be that dietary lipids tend to exert a greater influence on the fatty acid composition of the perirenal fat. Perhaps in the present study,

Table 8. Fatty acid content[1] of subcutaneous and perirenal fat.

	Diet A		Diet B		Diet C	
Fatty acid	Sub-cutaneous	Perirenal	Sub-cutaneous	Perirenal	Sub-cutaneous	Perirenal
12:0	0	0	0	0	0	0
14:0	1.2±1.7	0.9±0.7	1.1±0.2	0.9±0.1	1.6±1.4	1.0±0.3
16:0	15.2±1.2	14.8±1.6	16.1±0.9	16.9±0.7	18.1±3.8	15.9±1.7
16:1	6.1±1.2	5.2±1.8	8.0±2.2	5.3±1.0	6.0±0.8	4.7±2.6
18:0	3.0±0.7	3.4±0.7	3.0±1.5	3.8±1.2	7.5±7.7	4.7±2.1
18:1	35.0±1.3	34.6±2.2	33.4±1.3	34.1±3.0	34.1±1.6	33.9±2.0
18:2	34.3±1.6	34.7±2.9	33.3±1.0	39.3±1.3	29.3±10.5	35.2±4.1
18:3	5.0±2.0	4.9±3.2	3.6±1.6	4.5±2.4	3.1±0.8	4.0±1.1

[1]Appendix E and F.

the individual has been forced to withdraw saturated fatty acids from the lipid pools of the body in order to meet metabolic demands. The author has no other plausible explanation for the higher content of unsaturated fatty acids in the perirenal fat than in the subcutaneous fat.

Gordon et al. (1963) have previously reported results similar to those presented in the present study. They stated that the incorporation of a supplement of polyunsaturated fatty acids (40 percent dietary fat as

linoleic acid) in the diet of human subjects accelerated the rate of oxidation of saturated body fat by 20 to 25 percent.

Reiser et al. (1960) explained that in the presence of readily available carbohydrates, i.e. diet A in the present study, saturated fatty acids were utilized for energy to a greater degree than unsaturated fatty acids and therefore, are stored in lipid pools to a lesser extent.

The diets had no effect on the liver weight or lipid content as shown in Tables 9 and 10.

Table 9. Analysis of variance of liver weight[1] (g liver/100 g body weight)

Source	df	MS	F
Replicates	3	0.57	1.46
Diets	2	0.75	1.92
Replicates x diets	6	0.39	

[1]Appendix G.

Table 10. Analysis of variance of lipid content[1] of liver tissue (g/100 g wet weight)

Source	df	MS	F
Replicates	3	2.09	1.05
Diets	2	1.90	0.95
Replicates x diets	6	2.00	

[1]Appendix G.

The neutral lipids accounted for 19.39 ± 3.44 percent, 27.26 ± 11.68 percent and 34.61 ± 14.24 percent of the total liver lipids of rats on

diets A, B and C, respectively. The differences were not sufficient to prove significant as shown in Table 11.

Table 11. Analysis of variance of neutral lipid content[1] of rat liver lipids

Source	df	MS	F
Replicates	3	141.97	1.36
Diet	2	231.81	2.21
Replicates x diet	6	104.69	

[1]Appendix G

The liver cholesterol levels (mg/g) are given in Table 12.

Table 12. Cholesterol content of rat liver

	Mg/g liver		
Rat	Esterified	Free	Total
A1	8.32	15.45	23.77
2	8.94	12.28	21.22
3	3.66	15.49	19.15
4	2.53	23.74	26.27
B1	5.08	14.81	19.89
2	6.75	16.56	23.31
3	4.23	14.09	18.32
4	1.46	21.10	22.56
C1	1.92	7.70	9.62
2*	6.55	7.58	14.13
3	4.32	6.48	10.80
4	1.78	5.66	7.44

*Missing data supplied by method of Kempthorne (1952)

Statistical analysis of the liver cholesterol measurements (Table 13) indicated highly significant differences among diets and between forms (free and esterified).

Table 13. Analysis of variance of rat liver cholesterol measurements.

Source	df	MS	F
Replicates	3	3.34	0.83
Diet	2	86.59	21.54**
Replicates x diet	6	4.02	
Form	1	462.88	31.55**
Diet x form	2	47.51	3.24
Replicates x diet x form	9	14.67	

**$P < .01$

The dietary effects were further analyzed as shown in Table 14.

Table 14. Multiple range analysis of dietary effect on liver cholesterol levels

Standard error of dietary mean	± 2.71 (N_2 = 6)		
Number of pairs	(2)	(3)	
Shortest significant range	9.38	9.70	
Diet	C	B	A
Cholesterol level mg/g	10.50±2.79	21.02±2.32	22.60±3.09

Note: any two means not underscored by the same line are significantly different.

These results are in disagreement with those of Klein (1958), Avigan and Steinberg (1958), Russell et al. (1962), Garson et al. (1961) and Reiser et al. (1963) both as to the effect of the diet and as to the cholesterol content. These authors have reported that polyunsaturated fatty acids raise liver cholesterol values and give values in the range of 3 to 8 mg/g. The results in the present study agree with Alfin-Slater et al. (1954) and Mukherjee and Alfin-Slater (1958) in that liver cholesterol levels were decreased by feeding polyunsaturated fats as compared to fat free diets.

The present study is in general agreement with Jagannathan (1962a), (1962b), who reported that rat liver cholesterol concentrations were in the range of 13 to 21 mg/g. All rats used in these experiments were much younger than those used in the present experiment.

In view of the results presented here, the author is unable to concur with the hypothesis that the level of polyunsaturated fatty acids in the diet is the sole cause of the change in liver cholesterol concentrations. The author would agree that the increase in cholesterol concentrations is primarily in the free cholesterol fraction. The cholesterol ester concentrations of the rat livers were 5.86 ± 3.25 mg/g, 4.38 ± 2.20 mg/g and 3.64 ± 2.26 mg/g on diets A, B, and C, respectively, while the free cholesterol levels were 16.74 ± 4.90, 16.64 ± 3.16 and 6.86 ± 0.96 mg/g, respectively.

The fatty acid compositions of the fractions isolated from livers of rats fed diets A, B and C are given in Table 15, 16 and 17.

Table 15. Fatty acid composition of lipid fractions isolated from livers of rats fed diet A

Fatty acid	Triglyceride	Cholesterol esters	Diglyceride	Monoglyceride free fatty acids	Phospholipids
12:0	T	1.1±0.9	0.5±0.1	0.3±0.3	T
14:0	6.9±3.9	42.6±8.2	40.3±10.6	46.4±19.6	1.0±0.4
16:0	26.9±3.6	21.2±5.4	25.9±5.2	29.7±8.3	27.6±3.2
16:1	4.1±0.9	5.9±6.6	4.2±2.6	4.7±1.4	0
18:0	2.8±0.9	3.4±1.4	2.4±1.0	4.6±2.0	27.6±1.3
18:1	30.1±4.2	10.6±4.0	11.7±2.4	11.9±6.7	14.0±1.2
18:2	28.3±2.2	8.4±2.8	11.6±2.9	12.2±6.1	14.5±1.5
18:3	1.0±0.5	T	0.7±0.5	0.2±0.1	T
20:4	0.3±0.2	0	0	0	15.2±2.2

Table 16. Fatty acid composition of lipid fractions isolated from livers of rats fed diet B

Fatty acid	Triglyceride	Cholesterol esters	Diglyceride	Monoglyceride free fatty acids	Phospholipids
12:0	T	0.3±0.2	T	T	T
14:0	5.7±3.5	38.6±19.0	43.6±8.9	33.9±5.1	0.7±0.1
16:0	26.2±1.5	26.1±6.3	21.3±5.0	25.9±4.9	24.9±1.9
16:1	3.3±0.2	2.7±1.7	2.4±0.8	2.5±1.1	0
18:0	3.1±0.1	4.4±3.3	3.1±1.7	5.8±1.5	32.6±2.4
18:1	33.5±2.0	16.9±7.0	17.6±5.0	16.0±2.2	11.6±1.8
18:2	29.7±2.1	10.5±4.9	15.8±8.1	15.3±4.2	11.1±1.4
18:3	0.8±0.2	0.2±0.1	0.3±0.4	0.3±0.1	T
20:4	0	0	0	0	18.9±0.2

Table 17. Fatty acid composition of lipid fractions isolated from livers of rats fed diet C

Fatty acid	Triglyceride	Cholesterol esters	Diglyceride	Monoglyceride free fatty acids	Phospholipids
12:0	0	0.8±1.6	0	2.7±5.3	0
14:0	5.2±5.4	38.7±23.7	43.1±16.7	13.5±9.0	1.6±1.7
16:0	27.1±3.9	23.5±17.0	26.1±14.4	28.5±6.9	25.7±3.5
16:1	3.4±1.1	0.7±1.2	1.0±1.0	0.8±1.5	0
18:0	4.6±1.5	5.6±1.5	5.6±1.4	17.5±15.5	32.1±3.9
18:1	28.3±5.7	14.3±2.3	11.3±5.3	17.9±1.7	12.0±0.6
18:2	30.8±9.4	11.1±6.1	12.1±8.0	17.6±0.9	15.9±2.7
18:3	0.5±0.5	0.1±0.1	0.8±1.5	0.2±0.9	T
20:4	T	0	0	0	12.4±4.2

The statistical analysis of the polyunsaturated fatty acid composition of the various rat liver fractionsis given in Table 18.

Table 18. Analysis of variance of the polyunsaturated fatty acid composition[1] of lipid fractions isolated from livers of rats fed diets A, B and C.

Source	df	MS	F
Replicates	3	39.35	0.47
Diets	2	31.38	0.38
Replicates x diets	6	83.56	
Fractions	4	1044.15	53.06**
Diets x fractions	8	11.07	0.50
Replicate x diet x fraction	36	19.68	

**$P < .01$
[1]Appendices H, I, J, K and L.

The differences found among the fractionswere analyzed as shown in Table 19.

Table 19. Multiple range analysis of the polyunsaturated fatty acid content of fractions isolated from rat liver

Standard error of fraction mean			± 2.22 (N_2 = 36)		
Number of pairs:	(2)	(3)	(4)	(5)	
Shortest significant range:	8.52	8.88	9.15	9.28	
Fraction:	Cholesterol ester	Di-glyceride	Free fatty acid monoglyceride	Phospho-lipid	Tri-glyceride
Means:	10.13±4.36	13.72±6.40	15.60±5.01	29.31±4.12	30.27±5.57

Note: any two means not underscored by the same line are significantly different.

In view of the relatively small standard deviations presented for the triglyceride and phospholipid fatty acids from rat livers, the author would postulate that specific enzymatic processes apparently are responsible for the formation of these fractions. The hypothesis is strengthened by the lack of dietary effects on these fractions.

The similarities between the liver triglyceride and phospholipid polyunsaturated fatty acid content may support the triglyceride synthesis scheme proposed by Kennedy (1957). Geyer et al. (1960) reported that the fatty acid distribution on triglyceride molecules was similar to that of phospholipids in mouse fibroblasts.

The dietary effects on the blood serum lipid levels were non-significant in the present study (Table 20).

Table 20. Analysis of variance of blood serum lipid content[1] (mg/ml)

Source	df	MS	F
Replicates	2	69.88	0.60
Diets	2	335.58	2.86
Replicate x diet	4	117.21	

[1]Appendix M.

The serum lipid levels, 9.07 ± 0.82, 29.12 ± 14.56 and 24.94 ± 9.55 mg per ml, of rats on diets A, B and C, respectively, do indicate that high dietary lipid levels tend to increase serum lipids. Slaughter immediately after feeding may have produced significant effects in this case. The variance in the case of diets B and C may have been due to the differences

in elapsed time following the consumption of the ration by each rat before sacrifice.

The neutral lipids accounted for 55.61±5.87 percent, 10.40±6.12 percent and 7.97±4.78 percent of the total serum lipids of rats on diets A, B and C, respectively. These differences were highly significant as shown in Tables 21 and 22.

Table 21. Analysis of variance of neutral lipid content of rat blood serum lipids[1]

Source	df	MS	F
Replicates	2	45.94	2.18
Diets	2	2160.02	1020.37**
Replicate x diet	4	21.10	

**P < .01
[1]Appendix M.

Table 22. Multiple range analysis of dietary effects on proportion of neutral lipids in rat blood serum lipids.

Standard error of mean		± 6.50	
Number of pairs		(2)	(3)
Shortest significant range		25.55	26.07
Diet	C	B	A
Percent neutral lipid	7.97±4.78	10.40±6.12	55.61±5.87

Note: any two means not underscored by the same line are significantly different.

The blood serum cholesterol levels in mg/100 ml are presented in Table 23.

Table 23. Cholesterol content of rat blood serum

Rat	Total cholesterol mg	Free cholesterol mg/100 ml serum	Esterified cholesterol mg/100 ml serum
A 1	105.4	30.4	75.0
2	140.6	58.9	81.7
3	131.3	51.4	79.9
B 1	140.6	50.0	90.6
2	131.3	45.9	85.4
4	124.3	36.5	87.8
C 2	122.6	22.5	99.1
3	94.6	17.5	77.1
4	94.9	19.5	75.4

The analysis of variance of total blood serum cholesterol levels failed to show significant dietary effects (Table 24).

Table 24. Analysis of variance of rat blood serum cholesterol levels

Source	df	MS	F
Replicates	2	15.83	
Diets	2	335.35	1.86
Replicate x diet	4	180.35	
Form	1	9786.00	327.40**
Diet x form	2	397.05	13.28**
Replicate x diet x form	6	29.89	

**$P < .01$

Even in the presence of the diet-form interaction, the extremely high "F" test for form (free and esterified) indicates that a statistically significant difference exists between the free and esterified cholesterol levels. The diet-form interaction was investigated by analyzing the dietary effects on free and esterified cholesterol (Table 25).

Table 25. Analysis of variance of rat blood serum free cholesterol levels

Source	df	MS	F
Replicate	2	35.04	0.29
Diet	2	670.52	5.64
Replicate x diet	4	118.79	

Although there was not a statistical difference, it was apparent that the differences in the blood serum cholesterol level tended to occur in the free cholesterol fraction. The mean total cholesterol contents were 125.77 ± 18.26, 132.07 ± 8.17 and 103.60 ± 15.59 mg per 100 ml and esterified cholesterol contents were 78.87 ± 3.47, 87.93 ± 2.60 and 83.87 ± 8.65 mg per 100 ml in serum from rats on diets A, B and C, respectively.

These results disagree with those of Alfin-Slater et al. (1954) who reported that the dietary effects occurred in the esterified cholesterol fraction. It was further noted that there appears to be an inverse relationship between the phospholipid content of the blood serum lipids and cholesterol content. Okey and Lyman (1957) have reported a direct relationship. The inverse relationship was studied further by analyzing the variance of the phospholipid content of the rat blood serum (Table 26).

Table 26. Analysis of variance of the phospholipid content[1] of rat blood serum.

Source	df	MS	F
Replicate	2	46.66	0.42
Diet	2	439.18	3.95
Replicate x diet	4	111.13	

[1]Appendix M.

The diet had no significant effect on the phospholipid content on blood serum. However, examination of the dietary means reveals a trend in that the values were 3.99 ± 0.20, 26.61 ± 14.14 and 22.76 ± 8.31 mg per ml for diets A, B and C, respectively. These results also do not fit the hypothesis that there is a direct relationship between blood serum cholesterol and phospholipid levels (Okey and Lyman, 1957).

The fatty acid composition of the lipid fractions isolated from the blood serum of rats fed diets A, B and C is given in Tables 27, 28 and 29.

The statistical analysis of the polyunsaturated fatty acid compositions of these various fractions is given in table 30.

Table 30. Analysis of variance of the polyunsaturated fatty acid composition of lipid fractions[1] isolated from rat blood serum of rats fed diets A, B and C.

Source	df	MS	F
Replicates	2	1.58	0.04
Diet	2	59.89	1.57
Replicate x diet	4	38.04	
Fraction	4	196.10	13.64**
Diet x fraction	8	26.63	1.85
Replicate x diet x fraction	24	14.38	

**$P < .01$ [1]Appendices N, O, P, Q and R.

Table 27. Fatty acid composition of blood serum lipid fractions from rats on diet A.

Fatty acid	Triglyceride	Cholesterol ester	Diglyceride	Monoglyceride free fatty acid	Phospholipid
12:0	0.5 ± 0.2	1.0 ± 0.5	0.6 ± 0.4	0.8 ± 0.1	0.6 ± 0.6
14:0	16.0 ± 13.8	66.4 ± 10.3	53.2 ± 25.9	34.1 ± 10.3	8.0 ± 3.9
16:0	18.5 ± 4.8	11.2 ± 2.4	20.1 ± 11.0	29.1 ± 6.5	35.3 ± 3.2
16:1	5.2 ± 0.9	3.4 ± 2.1	2.6 ± 0.9	4.8 ± 1.1	7.4 ± 12.8*
18:0	8.9 ± 3.5	2.0 ± 0.7	5.8 ± 4.7	9.9 ± 2.8	25.9 ± 6.4
18:1	31.7 ± 7.5	7.5 ± 3.6	13.0 ± 7.3	16.8 ± 2.7	13.5 ± 3.0
18:2	6.6 ± 2.5	7.8 ± 6.2	3.3 ± 1.2	2.5 ± 2.8	6.4 ± 5.7
18:3	0.4 ± 0.4	0	0	0	T
20:4	0	0	0	0	2.4 ± 2.2

Rat A_1 had 22.1 percent palmitoleic acid in the phospholipid fraction.

Table 28. Fatty acid composition of blood serum lipid fractions from rats fed diet B.

Fatty acid	Triglyceride	Cholesterol ester	Diglyceride	Monoglyceride free fatty acid	Phospholipid
12:0	0.7 ± 0.0	2.0 ± 1.3	0.9 ± 0.5	0.6 ± 0.2	0.6 ± 0.5
14:0	59.0 ± 32.0	79.0 ± 2.4	43.6 ± 27.1	92.9 ± 26.0	7.3 ± 3.6
16:0	15.5 ± 11.6	6.6 ± 0.5	14.0 ± 1.9	25.9 ± 12.3	28.4 ± 2.0
16:1	4.1 ± 1.0	1.7 ± 1.4	2.9 ± 1.0	3.7 ± 0.6	0
18:0	5.0 ± 3.1	2.3 ± 0.9	4.5 ± 2.2	7.7 ± 6.6	30.4 ± 2.1
18:1	13.0 ± 10.8	2.7 ± 0.7	11.6 ± 2.8	14.5 ± 5.3	15.1 ± 0.9
18:2	6.3 ± 6.3	2.4 ± 0.7	3.7 ± 1.3	3.9 ± 2.3	14.2 ± 1.5
18:3	1.4 ± 2.0	0.2 ± 0.3	2.1 ± 2.8	0.4 ± 0.7	T
20:4	0	0	0	0	3.9 ± 3.1

Table 29. Fatty acid composition of blood serum lipid fractions from rats fed diet C.

Fatty acid	Triglyceride	Cholesterol ester	Diglyceride	Monoglyceride free fatty acid	Phospholipid
10:0	9.5 ± 9.1	0	4.1*± 10.0	0	0
12:0	2.0 ± 2.1	1.2 ± 0.8	6.5 ± 10.3	0.7 ± 0.4	1.2 ± 1.2
14:0	50.2 ± 12.6	64.1 ± 17.8	60.2 ± 20.7	60.1 ± 25.8	16.3 ± 6.1
16:0	11.5 ± 1.5	12.3 ± 6.3	9.0 ± 2.4	18.3 ± 9.8	21.5 ± 1.8
16:1	2.4 ± 0.6	1.8 ± 0.2	2.1 ± 1.2	1.8 ± 0.6	0.5**± 0.9
18:0	3.8 ± 1.1	3.3 ± 1.8	3.7 ± 4.1	3.9 ± 3.2	27.4 ± 0.2
18:1	11.6 ± 3.2	7.7 ± 6.1	6.5 ± 3.5	9.2 ± 8.2	11.5 ± 1.7
18:2	8.0 ± 3.2	5.0 ± 8.6	7.7 ± 3.1	5.8 ± 4.1	14.3 ± 2.3
18:3	0.5 ± 0.8	0	0	0.3 ± 0.6	T
20:4	0	0	0	0	7.2 ± 3.8

* 12.3% capric acid in C_1
**1.5% palmitoleic in C_4

The multiple range analysis of the polyunsaturated fatty acid content of the fractions is given in Table 31.

Table 31. Multiple range analysis of the polyunsaturated fatty acid content of rat serum lipid fractions.

Standard error of fraction mean	± 1.89 (N_2 = 24)				
Pairs	(2)	(3)	(4)	(5)	
Shortest significant range	7.48	7.82	8.01	8.18	
Fraction:	Monoglyceride free fatty acid	di-glyceride	Cholesterol ester	Tri-glyceride	Phospho-lipid
Percent polyunsaturated fatty acid	4.66±2.95	5.59±2.80	5.98±4.47	7.69±3.75	16.12±5.08

Note: any two means not underscored by the same line are significantly different.

These results indicate that the phospholipid fraction of rat blood serum contains significantly more polyunsaturated fatty acids. Geyer et al. (1960) have previously reported that the fatty acid distribution patterns of triglycerides and phospholipids in human blood serum were dissimiliar.

The fatty acid composition of the pooled aorta samples from each dietary group is presented in Table 32.

Table 32. Fatty acid composition of triglycerides, cholesterol esters, monoglyceride-free fatty acids and phospholipids isolated from pooled aorta samples.

Fraction	Diet	Fatty acid composition 12:0	14:0	16:0	16:1	18:0	18:1	18:2	18:3	20:4
Triglyceride	A	0.1	3.1	24.9	5.7	5.1	33.9	27.1	0.2	0
	B	0.1	2.4	20.3	4.0	8.4	36.7	26.7	1.4	0
	C	0.1	6.6	18.8	3.8	8.3	27.9	32.7	1.9	0
Cholesterol	A	0.6	23.8	20.5	3.4	5.8	25.9	18.7	1.0	0
esters	B	0.3	12.1	18.7	1.9	10.3	34.6	22.0	0.5	0
	C	1.2	20.9	20.5	0	12.5	22.3	20.9	1.7	0
Monoglyceride	A	0.1	6.7	20.3	2.7	20.7	22.9	26.6	0	0
-free fatty	B	0.2	11.4	16.4	0	21.4	23.2	26.4	T	0
acids	C[1]	0.3	7.3	18.4	0	19.4	23.1	27.6	0.3	0
Phospholipids	A	0.1	2.9	20.9	3.3	9.8	28.1	32.8	1.4	0.7
	B	T	6.1	18.3	5.2	12.1	32.7	24.9	1.1	0.5
	C	0.1	4.3	17.2	2.6	10.2	28.2	36.0	0.9	0.1

[1]3.6 percent 20:3 was detected.

The analysis of variance indicated no significant effects due to diet or fraction as shown in Table 33.

Table 33. Analysis of variance of the polyunsaturated fatty acid content[1] of lipid fractions isolated from aortas of rats fed diets A, B and C.

Source	df	MS	F
Diet	2	8.76	0.62
Fraction	3	44.13	3.10
Diet x fraction	6	14.23	

The fractions tended to have different contents of polyunsaturated fatty acids. The mean values were 30.0 ± 4.0, 29.1 ± 5.0, 28.3 ± 2.8 and 21.6 ± 1.6 for the triglyceride, phospholipid, and monoglyceride-free fatty acid and cholesterol ester fractions, respectively.

The polyunsaturated fatty acid content of triglyceride fractions varied from tissue to tissue as shown in Tables 34 and 35.

Table 34. Analysis of variance of the polyunsaturated fatty acid content of triglyceride from rat blood, liver and aorta.

Source	df	MS	F
Replicates	2	16.09	1.09
Diet	2	23.87	1.62
Replicate x diet	4	14.70	
Tissue	2	1454.80	64.37**
Diet x tissue	4	16.89	0.75
Replicate x diet x tissue	12	22.60	

**$P < .01$

Table 35. Multiple range test of polyunsaturated fatty acid content of triglyceride from rat blood, liver and aorta.

Standard error of location		$\pm$ 3.40	(N_2 = 12)
Number of pairs	(2)	(3)	
Shortest significant range	10.47	10.98	
Tissue:	Blood	Liver	Aorta
Percent polyunsatured fatty acid	7.69 ± 3.75	29.41 ± 5.77	30.00 ± 3.46

Note: any two means not underscored by the same line are significantly different.

Reiser et al. (1960) reported that plasma triglycerides did not originate in the livers of miniature pigs. This conclusion was supported by the present study involving rats. Apparently, triglycerides with higher levels of polyunsaturated fatty acids were either selectively retained by the liver or deposited in the aorta wall.

The differences in the unsaturated fatty acid content of cholesterol esters from various tissues are shown in Tables 36 and 37.

Table 36. Analysis of variance of the unsaturated fatty acid content of rat liver, blood and aorta cholesterol esters.

Source	df	MS	F
Replicates	2	18.31	0.19
Diet	2	12.48	0.13
Replicate x diet	4	97.86	
Tissue	2	3138.93	50.73**
Diet x tissue	4	139.69	2.26
Replicate x diet x tissue	12	61.68	

**P < .01

Table 37. Multiple range test for unsaturated fatty acid content of the cholesterol esters of rat blood, liver and aorta.

Standard error of tissue mean		± 5.56	(N_2 = 12)
Number of pairs	(2)	(3)	
Shortest significant range	19.20	19.86	
Tissue	Blood	Liver	Aorta
Percent unsaturated fatty acid	14.81±9.42	24.64±9.67	50.93±6.06

Note: any two means not underscored by the same line are significantly different.

These results are in contrast to Swell et al. (1960b), who reported similarities between the fatty acid composition of cholesterol esters in human serum and aortas. Swell et al. (1960a) reported distinctive differences between rat serum and liver cholesterol esterified fatty acids. The present study indicated non-significant differences between these tissues.

The present work supports the conclusion of Evrard et al. (1962) that the cholesterol esterified fatty acid pattern of the serum differs from that of the aorta. The present study also supports the report of Reiser et al. (1960) in that significant differences were not present between the cholesterol esterified fatty acid patterns of blood serum and liver. Swell et al. (1964) reported that liver cholesterol esters bear no relationship to blood serum cholesterol esters.

Table 38 indicates that significant differences exist in the polyunsaturated fatty acid content of rat blood serum and liver diglycerides. The blood serum contained 5.59+3.22 percent and liver, 15.28$\pm$5.68 percent polyunsaturated fatty acids.

Table 38. Analysis of variance of the polyunsaturated fatty acid content of rat liver and blood diglycerides.

Source	df	MS	F
Replicates	2	5.92	0.35
Diet	2	28.08	1.67
Replicate x diet	4	16.82	
Tissue	1	422.44	14.30**
Diet x tissue	2	14.16	0.48
Replicate x diet x tissue	6	29.55	

**$P < .01$

Significant differences were found between the polyunsaturated fatty acid content of the monoglyceride-free fatty acid fraction isolated from various rat tissues (Tables 39 and 40).

Table 39. Analysis of variance of the polyunsaturated fatty acid content of monoglyceride-free fatty acid fraction of rat liver, blood and aorta.

Source	df	MS	F
Replicates	2	13.35	0.29
Diet	2	41.29	.91
Replicate x diet	4	45.49	
Tissue	2	1258.47	1006.67**
Diet x tissue	4	1.85	1.48
Replicate x diet x tissue	12	1.25	

**P < .01

Table 40. Multiple range test for polyunsaturated fatty acid content of the monoglyceride-free fatty acid fraction of rat blood, liver and aorta.

Standard error of tissue mean		± 0.79	(N_2 = 12)
Number of pairs	(2)		(3)
Shortest significant range	2.43		2.55
Tissue	Blood	Liver	Aorta
Percent polyunsaturated fatty acids	4.66±2.95	16.04±4.96	28.30±2.40

Note: any two means not underscored by the same line are significantly different.

Significant differences were found in the polyunsaturated fatty acid content of the phospholipids isolated from rat serum, liver and aorta (Tables 41 and 42).

Table 41. Analysis of variance of the polyunsaturated fatty acid content of phospholipids isolated from the serum, livers and aortas of rats fed diets A, B and C.

Source	df	MS	F
Replicates	2	2.58	0.34
Diets	2	37.87	5.04
Replicate x diet	4	7.51	
Tissue	2	681.73	33.30**
Diet x tissue	4	99.42	4.86*
Replicate x diet x tissue	12	20.47	

*$P < .05$, **$P < .01$

Table 42. Multiple range test for polyunsaturated fatty acid content of the phospholipid fractions isolated from rat serum, liver and aortas.

Standard error of mean		$\pm$ 3.20 (N_2 = 12)	
Number of pairs	(2)	(3)	
Shortest significant range	9.86	10.18	
Tissue	Blood serum	Liver	Aorta
Percent polyunsaturated fatty acids	16.12$\pm$7.14	28.78$\pm$4.50	32.80$\pm$4.81

Note: any two means not underscored by the same line are significantly different.

Dietary lipids evidently had a limited effect on the polyunsaturated fatty acid composition of the phospholipid fraction. Means for the effects of diets A, B and C were 24.6, 24.8 amd 28.3 percent polyunsaturated fatty acids. Based on the scheme of phospholipid synthesis from α, β diglycerides proposed by Kennedy and Weiss (1955), (1956), it is possible to explain the presence of a greater percentage of polyunsaturates in the phospholipids isolated from rats on diet C. This explanation has also been suggested by Lands and Hart (1964).

Brockerhoff et al. (1964) fed glyceryl 1, 3 dioleate, 2 palmitate -1-C^{14} to rats and found no relationship between this structure and that of palmitate -1-C^{14} labeled triglycerides and lecithin. The author would suggest that in the presence of specific enzymes for phospholipid synthesis, the β-unsaturated triglyceride supplied by diet C would be more readily acceptable as a precursor for phospholipid synthesis.

It has been shown in the present study that the blood serum lipids contained a higher percentage of saturated fatty acids than did the aorta or liver lipids. Fisher and Gurin (1964) reported that high density (> 1.063 and < 1.21) plasma lipoprotein isolated from rat serum contained small quantities of firmly bound long-chain saturated fatty acids. These authors reported that these fatty acids are in all probability, covalently bonded to the protein.

In a general review of protein lipid interactions and their relation to the physical-chemical stability of concentrated milk, Brunner (1962) was careful not to draw the apparent conclusion that the bound lipid molecule associates itself only with receptive sites in the protein. If this were the case, the existence of low-density lipoproteins, such as the

β-lipoprotein of blood plasma, in which the lipid moiety constitutes more than 70 percent of the complex could not be explained. Brunner (1962) explained that in this case, most of the lipid molecules find themselves in association with other lipid molecules. The hydrocarbon moieties of these lipids interact strongly through nonpolar bonding, i.e. van der Waals' forces. Long chain saturated fatty acids, such as stearic, pack rather closely, whereas the distortion around the double bonds of unsaturated fatty acids precludes close packing. In the presence of saturated fatty acids, a more cohesive complex could then be formed. Wolf and Dugan (1964) reported that the milk fat-globule membrane contained 89.3 percent saturated fatty acids in the triglyceride fraction, 91.1 percent in the diglyceride fraction and 84.5 percent in the free fatty acid fraction. Calculations revealed that 71.2 percent of the triglycerides were trisaturated. Mono- and di-unsaturated glycerides contained saturates primarily at the β-ester. The author would then assume that the trisaturated or β-saturated glycerides are required to form stable complexes.

Gurd (1960) reported that lipid components are exchanged or transferred from the lipoprotein complexes by actual collision between lipoprotein complexes themselves. The exchange or transfer occurs in the resulting collision complex which then decomposes once more into individual lipoprotein complexes.

Downie et al. (1963) have shown that lipid deposits occur at the bifurcation of arteries. At these points there would be a greater physical stress on the lipoprotein complexes as described by Gurd (1960). The present study indicates that rat serum contains significantly more saturated

fatty acids in all of the lipid fractions. This leads the author to the conclusion that lipid fractions containing higher contents of polyunsaturated fatty acids form less stable lipoprotein complexes in the serum. These lipid moieties are deposited through physical processes in the more stationary tissues. The accumulation of polyunsaturated fatty acids in various tissues is then predicted to occur. Furthermore, an explanation is possible for the higher cholesterol values observed in blood serum of subjects fed animal fats. More stable lipoprotein complexes would be formed due to the higher saturated fatty acid content and to the β-saturated glycerides found in these fats. The stable lipoprotein complexes would be less likely to release cholesterol and cholesterol esters in the liver where they could be converted to and excreted as bile acids.

SUMMARY AND CONCLUSIONS

A study was made to determine the effects of dietary triglyceride structure on various lipid components of rat tissues and feces. A lipid containing 33 percent polyunsaturated fatty acids predominantly at the α or α' ester linkage was isolated from lard and was incorporated into diet B. Another lipid fraction, containing 33 percent polyunsaturated fatty acids predominantly at the β-ester linkage, was fractionally crystallized from soybean oil. This lipid was introduced into diet C. These effects were compared with those of a fat free diet (diet A).

Inclusion of lipids at the level of 40 percent of the calories significantly increased the feces lipid levels. The diets had no significant effect on the polyunsaturated fatty acid content of the feces neutral lipid classes. However, the polyunsaturated fatty acid content of feces from rats fed diet C tended to be higher. Apparently, there was selective absorption of polyunsaturated fatty acids at the expense of palmitic and stearic acids. There were indications that the phospholipid content of the feces from rats fed diet B was somewhat elevated.

The diets produced highly unsaturated body fats. It was postulated that a deficient supply of saturated fatty acids in the diet forced the rats to utilize saturated fatty acids previously deposited in the body fat deposits.

Diet C produced significantly lower liver cholesterol values. This decrease occurred in the free cholesterol fraction.

The polyunsaturated fatty acid content of liver triglycerides and phospholipids was significantly higher than in the other fractions.

Indirect evidence led to the hypothesis that there were specific enzymes involved in the synthesis of these fractions. The levels of dietary lipids tended to have a direct relationship to the level of blood lipids. The phospholipid content of the serum lipids from rats fed diets B and C was significantly higher. This trend was also indicated by the apparent increase in the phospholipid content of blood serum from rats fed diets B and C. The phospholipids contained a significantly higher proportion of polyunsaturated fatty acids than did the other serum lipid fractions.

Blood serum cholesterol levels were decreased by feeding diet C. This decrease occurred in the free cholesterol fraction. The lipid fractions of the aorta samples tended to be similar to those of the liver.

In view of these results, it was postulated that fractions containing the more unsaturated fatty acids form less stable lipoprotein complexes in the blood serum. These complexes would be more likely to decompose under physical stress allowing for the transfer of lipid fractions to the more stationary tissues, i.e. liver and aorta. It was further postulated that the lipid structure containing the unsaturates primarily at the β-ester would be less likely to serve as a precursor for the trisaturates and the β-saturated mono- and di-saturated glycerides that tend to stabilize lipoprotein complexes. This hypothesis would explain the different effects of diets B and C on blood cholesterol even though they contained equal amounts of polyunsaturated fatty acids. In the case of diet B, more stable lipoprotein complexes would be formed and the level of circulating cholesterol would be elevated as compared to diet C. This may offer a clue to the cholesterol-lowering effects of vegetable oils.

BIBLIOGRAPHY

Aftergood, L., H. J. Deuel, Jr., and R. B. Alfin-Slater. 1956. The comparative effects of cottonseed oil and lard on cholesterol levels in the tissues of rats. J. Nutr. 62:129.

Ahrens, E. H. Jr., R. Blomstrand, J. Hirsch, W. Insull, Jr., M. L. Peterson and T. T. Tsaltas. 1957. The influence of dietary fats on serum lipide level in man. Lancet 272:943.

Ahrens, E. H. Jr., J. Hirsch, W. Insull, Jr., R. Blomstrand, T. T. Tsaltas and M. L. Peterson. 1957. The influence of dietary fats on serum lipide levels in man. Jour. Amer. Oil Chemists Soc. 34:563.

Ahrens, E. H. Jr., C. H. Blankenhorn and T. T. Tsaltas. 1954. Effect on human serum lipids of substituting plant for animal fat in the diet. Proc. Soc. Exp. Biol. Med. 86:872.

Alfin-Slater, R. B., L. Aftergood, A. F. Wells and H. J. Deuel, Jr. 1954. The effect of essential fatty acid deficiency on the distribution of endogenous cholesterol in the plasma and liver of the rat. Arch. Biochem. Biophys. 52:180.

Avigan, J. and D. Steinberg. 1958. Effect of saturated and unsaturated fat on cholesterol metabolism in the rat. Proc. Soc. Exp. Biol. Med. 97:814.

Bates, M. W. 1958. Turnover rates of fatty acids of plasma triglycerides, cholesterol ester and phospholipid in postabsorptive dog. Am. J. Physiol. 194:446.

Benson, J. A., G. N. Chandler, F. E. Vansteenhuyse and J. O. Gagnon. 1956. Studies concerning the site of fat absorption in the small intestine of the rat. Gastroenter. 30:53.

Brockerhoff, H., R. J. Hoyle and K. Ronald. 1964. Retention of the fatty acid distribution pattern of a dietary triglyceride in animals. J. Biol. Chem. 239:735.

Bronte-Stewart, B., A. Antonis, L. Eales and J. F. Brock. 1956. Effects of feeding different fats on serum cholesterol level. Lancet 270:521.

Brot, N., W. J. Lossow and I. L. Chaikoff. 1964. In vitro uptake and hydrolysis, by rat tissue, of cholesterol esters of a very low density, chyle lipoprotein fraction. J. Lipid Res. 5:63.

Brown, H. B. and I. H. Page. 1958. Lowering blood lipid levels by changing food patterns. J. Amer. Med. Assn. 168:1989.

Brozek, J., R. Buzina and F. Mikic. 1957. Population studies on serum cholesterol and dietary fat in Yugoslavia. Am. J. Clin. Nutr. 5:279.

Brunner, J. R. 1962. Protein-lipid interactions and their relation to the physical-chemical stability of concentrated milk. A review. J. Dairy Sci. 45:943.

Buell, G. C. and R. Reiser. 1959. Glyceride-glycerol precursors in the intestinal mucosa. J. Biol. Chem. 234:217.

Carroll, K. K. 1961. Separation of lipid classes by chromatography on florisil. J. Lipid Res. 2:135.

Choudhury, R. B. R. and L. K. Arnold. 1960. The determination of the neutral oil content of crude vegetable oils. J. Am. Oil Chemists' Soc. 37:87.

Clark, B. and G. Hübscher. 1961. Biosynthesis of glycerides in subcellular fractions of intestinal mucosa. Biochim. Biophys. Acta 46:479.

Clark, B. and G. Hübscher. 1960. Biosynthesis of glycerides in the mucosa of the small intestine. Nature 185:35.

Desnuelle, P., M. Naudet and J. Rouzier. 1948. Etude quantitative de la formation de glycerides partiels au cours de l'hydrolyse fermentaire des triglycerides. Biochim. Biophys. Acta 2:561.

Deuel, H. J. Jr. 1955. The Lipids. Vol. II Biochemistry, Digestion, Absorption, Transport, Storage. Interscience Publishers, Inc., New York.

Dittmer, J. C. and D. J. Hanahan. 1959. Biochemistry of long chain fatty acids. I. General observations. J. Biol. Chem. 234:1976.

Dole, V. P., A. T. James, J. P. W. Webb, M. A. Rizack and M. F. Sturman. 1959. The fatty acid patterns of plasma lipids during alimentary lipemia. J. Clin. Invest. 38:1544.

Downie, H. G., E. A. Murphy, H. C. Rowsell and J. F. Mustard. 1963. Extra corporeal circulation: A device for the quantitative study of thrombus formation in flowing blood. Circulation Res. 12:441.

Dugan, L. R. Jr., and G. W. McGinnis. 1964. Direct conversion to methyl esters of fatty acids in biological materials. In press.

Duncan, D. B. 1955. The multiple range and multiple F-test. Biometrics 11:1.

Evrard, E., J. Van Den Bosch, P. De Somer and J. V. Joossens. 1962. Cholesterol ester fatty acid patterns of plasma, atheroma and livers of cholesterol-fed rabbits. J. Nutr. 76:219.

Fisher, W. R. and S. Gurin. 1964. Structure of lipoproteins: Covalently bound fatty acids. Science 143:362.

Frazer, A. C. 1962. The fate of dietary fat in the body. Chem. Ind. (London) 1962:1438.

Frazer, A. C. 1960. Emulsions in vivo. Lab. Pract. 9:847.

Frazer, A. C. and H. G. Sammons. 1945. The formation of mono- and diglycerides during the hydrolysis of triglyceride by pancreatic lipase. Biochem. J. 39:122.

Gerson, T., F. B. Shoreland and Y. Adams. 1961. The effects of corn oil on the amounts of cholesterol and the excretion of sterol in the rat. Biochem. J. 81:584.

Getz, G. S., W. Bartley, F. Stirpe, B. M. Notton and A. Renshaw. 1961. The lipid composition of rat liver. Biochem. J. 80:176.

Geyer, R. P., A. Bennett and A. Rohr. 1962. Fatty acids of the triglycerides and phospholipids of HeLa cells and strain L. fibroblasts. J. Lipid Res. 3:80.

Gidez, L. I., R. S. Roheim and H. A. Eder. 1962. Effect of plasma free fatty acid concentration on triglyceride synthesis by the perfused liver. Federation Proc. 21:289 (abstract)

Glomset, J. A., F. Parker, M. Tjaden and R. H. Williams. 1962. The esterification in vitro of free cholesterol in human and rat plasma. Biochim. Biophys Acta 58:398.

Gordon, E. S., M. Goldberg and G. J. Chosy. 1963. A new concept in the treatment of obesity. J. Am. Med. Assn. 186:50.

Gurd, R. R. N. 1960. Some naturally occurring lipoprotein systems. In Lipide Chemistry (D. J. Hanahan ed.) John Wiley and Sons, Inc. New York. 260.

Hanahan, D. J., H. Brockherhoff and E. J. Barron. 1960. The site of attack of phospholipase (lettinase) A on lecithin: A re-evaluation. J. Biol. Chem. 235:1917.

Hardinge, M. G. and F. J. Stare. 1954. Nutritional studies of vegetarians: Dietary and serum levels of cholesterol. Am. J. Clin. Nutr. 2:83.

Hilditch, T. P. and W. J. Stainsby. 1935. XII. The body fats of the pig. IV Progressive hydrogenation as an aid in the study of glyceride structure. Biochem. J. 29:90.

Hoet, P. P., J. V. Joossens, E. Evrard, H. Eyssen and P. De Sommer. 1963. Intestinal bacteria and faecal fat. In Biochemical Problems of Lipids. (A. C. Frazer, ed.) Elsevier Publishing Co., New York. 173.

Horlick. L. and B. M. Craig. 1957. Effect of long-chain polyunsaturated and saturated fatty acids on the serum lipids of man. Lancet 273:566.

Jacobson, G. A., P. J. Tienstra and W. D. Pohle. 1961. The differential cooling curve. A technique for measuring certain fat characteristics. J. Am. Oil Chemists' Soc. 38:399.

Jagannathan, S. N. 1962a. Effect of feeding fat blends of hydrogenated groundnut (peanut) fat and cottonseed oil containing different levels of linoleic acid on serum cholesterol levels in monkeys (Macaca radiata) and liver cholesterol concentration in cholesterol-fed rats. J. Nutr. 77:317.

Jagannathan, S. N. 1962b. Effect of feeding different dietary fat mixtures providing the same amount of linoleic acid, on serum cholesterol levels in monkeys and liver cholesterol concentration in cholesterol-fed rats. J. Nutr. 77:323.

James, A. T., J. P. W. Webb and T. D. Kellock. 1961. The occurrence of unusual fatty acids in faecal lipids from human beings with normal and abnormal fat absorption. Biochem. J. 78:333.

Jolliffe, N., S. H. Rinzler and M. Archer. 1959. The anti-coronary club; Including a discussion of the effects of a prudent diet on the serum cholesterol level of middle-aged men. Amer. J. Clin. Nutr. 7:45.

James, A. T. and J. E. Lovelock. 1958. Essential fatty acids and human disease. Brit. Med. Bull. 14:262.

Karmen, A., M. Whyte and D. S. Goodman. 1963. Fatty acid esterification and chylomicron formation during fat absorption: 1. Triglycerides and cholesterol esters. J. Lipid Res. 4:312.

Kempthorne, O. 1952. The Design and Analysis of Experiments. John Wiley and Sons, Inc. New York.

Kennedy, E. P. 1957. Metabolism of lipides. Ann. Rev. Biochem. 26:119.

Kennedy, E. P. and S. B. Weiss. 1956. The function of cytidine coenzymes in the biosynthesis of phospholipids. J. Biol. Chem. 222:193.

Kennedy, E. P. and S. B. Weiss. 1955. Cytidine diphosphate choline: A new intermediate in lecithin biosynthesis. J. Am. Chem. Soc. 77:250.

Keys, A., J. T. Anderson, M. Aresu, G. Biorck, J. F. Brock, B. Bronte-Stewart, F. Fidanza, M. H. Keys, H. Malmos, A. Poppi, T. Postili, B. Swahn and A. del Vecchio. 1956. Physical activity and diet in populations differing in serum cholesterol. J. Clin. Invest. 35: 1173.

Kinsell, L. W., J. Partridge, L. Boling, S. Marger and G. Michaels. 1952. Dietary modification of serum cholesterol and phospholipide levels. J. Clin. Endrocrinol. Metab. 12:909. (Letter to editor).

Kleiber, M. 1961. The Fire of Life an Introduction to Animal Energetics. John Wiley and Sons Inc. New York.

Klein, P. D. and E. R. Janssen. 1959. The fractionation of cholesterol esters by silicic acid chromatography. J. Biol. Chem. 234:1417.

Klein, P. D. and R. A. Martin. 1959. Heterogeneity in the turnover rates of liver cholesterol esters. J. Biol. Chem. 234:1685.

Klein, P. D. 1958. Linoleic acid and cholesterol metabolism in the rat. I. The effect of dietary fat and linoleic acid levels on the content and composition of cholesterol esters in liver and plasma. Arch. Biochem. Biophys. 76:56.

Lands, W. E. M. and P. Hart. 1964. Metabolism of glycerolipids. V. Metabolism of phosphatidic acid. J. Lipid Res. 5:81.

Linazasoro, J. M., R. Hill, F. Chevalier and I. L. Chaikoff. 1958. Regulation of cholesterol synthesis in the liver: The influence of dietary fats. J. Exptl. Med. 107:813.

Macfarlane, M. G., G. M. Gray and L. W. Wheeldon. 1960. Fatty acid composition of phospholipids from subcellular particles of rat liver. Biochem. J. 77:626.

Mangold, H. K. 1961. Thin-layer chromatography of lipids. J. Am. Oil Chemists' Soc. 38:708.

Marinetti, G. V., J. Erbland and E. Stotz. 1960. Hydrolysis of lecithins by venom phospholipase A II. Fatty acid chain length preference of the enzyme. Biochim. Biophys. Acta 38:534.

Mattson, F. H., R. A. Volpenheim and E. S. Lutton. 1964. The distribution of fatty acids in the triglycerides of the artiodactyla. J. Lipid Res. 5:363.

Mattson, F. H. and R. A. Volpenheim. 1963. The specific distribution of unsaturated fatty acids in the triglycerides of plants. J. Lipid Res. 4:392.

Mattson, F. H. and L. W. Beck. 1956. The specificity of pancreatic lipase for the primary hydroxyl groups of glycerides. J. Biol. Chem. 219: 735.

Mattson, F. H., J. H. Benedict, H. B. Martin and L. W. Beck. 1952. Intermediates formed during the digestion of triglycerides. J. Nutr. 48:335.

McGinnis, G. W. and L. R. Dugan, Jr. 1964. A rapid low-temperature method for preparation of methyl esters of fatty acids. In Press.

Mead, J. F. 1957. The metabolism of the essential fatty acids. VI. Distribution of unsaturated fatty acids in rats on fat-free and supplemented diets. J. Biol. Chem. 227:1025.

Mohrhauer, H. and R. T. Holman. 1963a. The effect of dose level of essential fatty acids upon fatty acid composition of the rat liver. J. Lipid Res. 4:151.

Mohrhauer, H. and R. T. Holman. 1963b. The effect of dietary essential fatty acids upon composition of polyunsaturated fatty acids in depot fat and erythrocytes of the rat. J. Lipid Res. 4:346.

Mukherjee, S., K. T. Achaya, H. J. Deuel, Jr., and R. B. Alfin-Slater. 1958. The effect of dietary fat on the fatty acid composition of cholesterol esters in rat liver. J. Nutr. 65:469.

Mukherjee, S. and R. B. Alfin-Slater. 1958. The effect of the nature of dietary fat on synthesis of cholesterol from acetate-1-C^{14} in rat liver slices. Arch Biochem Biophys. 73:359.

Mukherjee, S., K. I. Achaya, H. J. Deuel, Jr., and R. B. Alfin-Slater. 1957. The nature of the lipides in rat blood. J. Biol. Chem. 226: 845.

Nestel, P. J. and D. Steinberg. 1963. Fate of palmitate and of linoleate perfused through the isolated rat liver at high concentrations. J. Lipid Res. 4:461.

Nichaman, M. Z., C. C. Sweeley, N. M. Oldham and R. E. Olson. 1963. Changes in fatty acid composition during preparative thin-layer chromatography. J. Lipid Res. 4:484.

Okey, R., R. Otswald, A. Shannon and J. Tinoco. 1962. Changes in tissue lipids in response to diet. II. Fatty acid composition of liver and plasma lipids in relation to fed and stored fat. J. Nutr. 76:353.

Okey, R. and M. M. Lyman. 1957. Dietary fat and cholesterol metabolism. I. Comparative effects of coconut and cottonseed oils at three levels of intake. J. Nutr. 61:523.

Ostrander, J. and L. R. Dugan, Jr. 1962. Some differences in composition of covering fat, intermuscular fat and intramuscular fat of meat animals. J. Am. Oil Chemists' Soc. 39:178.

Patil, V. S. and N. G. Magar. 1960. Effect of dietary fat and cholesterol on the polyunsaturated fatty acids of cholesterol esters, phospholipids and triglycerides in the liver of the rat. Biochem. J. 74:441.

Pollak, O. J. 1959. Diet and atherosclerosis -- Variations on a theme. Am. J. Clin. Nutr. 7:502.

Rabinowitz, J. L., R. M. Myerson and G. T. Wohl. 1960. Deposition of C^{14} labelled cholesterol in the atheromatous aorta. Proc. Soc. Exp. Biol. Med. 105:241.

Reiser, R., M. C. Williams, M. F. Sorrels and M. L. Murty. 1963. Biosynthesis of fatty acids and cholesterol as related to diet fat. Arch. Biochem. Biophys. 102:276.

Reiser, R., M. C. Williams and M. F. Sorrels. 1960. Tissue lipide relationships from their composition and acetate incorporation. Arch. Biochem. Biophys. 86:42.

Reiser, R., M. F. Sorrels and M. C. Williams. 1959. Influence of high levels of dietary fats and cholesterol on atherosclerosis and lipid distribution in swine. Circulation Res. 7:833.

Rose, H., M. Vaughan and D. Steinberg. 1964. Utilization of fatty acids by rat liver slices as a function of medium concentration. Am. J. Physiol. 206:345.

Russell, P. T., J. C. Scott and J. T. Van Bruggen. 1962. Effects of dietary fat on cholesterol metabolism in the diabetic rat. J. Nutr. 76: 460.

Schoenheimer, R. and W. M. Sperry. 1934. Sterol secretion and coprosterol formation. J. Biol. Chem. 107:1.

Shoreland, F. B., R. O. Weenink, A. T. Johns and I. T. R. McDonald. 1957. Hydrogenating action of sheep-rumen contents. Biochem. J. 67:328.

Siperstein, M. D. and I. L. Chaikoff. 1952. C^{14}-cholesterol. III. Excretion of carbons 4 and 26 in feces, urine and bile. J. Biol. Chem. 198:93.

Snedecor, G. W. 1956. Statistical Methods 5th ed. The Iowa State College Press, Ames, Iowa.

Spitz, N. and E. H. Ahrens, Jr. 1963. Direct measurement of sterol and bile acids in human feces. In Biochemical Problems of Lipids. (A. C. Frazer, ed.) Elsevier Publishing Co., New York. 66.

Swell, L., M. D. Law and C. R. Treadwell. 1964. Esterification of cholesterol in rat liver microsomes. Arch. Biochem. Biophys. 104:128.

Swell, L., M. D. Law and C. R. Treadwell. 1962. Tissue cholesterol ester and triglyceride fatty acid composition of rabbits fed cholesterol diets high and low in linoleic acid. J. Nutr. 76:429.

Swell, L., H. Field, Jr., P. E. Schouls, Jr. and C. R. Treadwell. 1960a. Fatty acid composition of tissue cholesterol esters in elderly humans with atherosclerosis. Proc. Soc. Exp. Biol. Med. 103:651.

Swell, L., H. Field, Jr., P. E. Schouls, Jr. and C. R. Treadwell. 1960b. Lipid fatty acid composition of several areas of the aorta in subjects with atherosclerosis. Proc. Soc. Exp. Biol. Med. 105:662.

Swell, L., M. D. Law, H. Fields, Jr., and C. R. Treadwell. 1960c. Cholesterol ester fatty acids in serum and liver of normal and lymph-fistula rats. J. Biol. Chem. 235:1960.

Tattrie, N. H. 1959. Positional distribution of saturated and unsaturated fatty acids on egg lecithin. J. Lipid Res. 1:60.

Wagner, R. G. 1962. Nutrient requirement of the laboratory rat. In Nutrient Requirements of Domestic Animals. No. X. Nutrient Requirements of Laboratory Animals. National Academy of Sciences, National Research Council Publication 990. Washington, D. C.

Webb, J. P. W., A. T. James and T. D. Kellock. 1961. The effects of changes in dietary fats on the fatty acid composition of faecal lipids from subjects with normal and abnormal fat absorption. In Digestion, Absorption, Intestinale et Transport Des Glycerides Chez les Animaux Superieurs. Editions Du Centre National De la Recherche Scientifique. Paris. 113.

Weinhouse, S. and E. F. Hirsch. 1940. Chemistry of Atherosclerosis I. Lipid and calcium content of the intima and of the media of the aorta with and without atherosclerosis. Arch. Path. 29:31.

Wolf, D. P. and L. R. Dugan, Jr. 1964. Structure of high melting glycerides from the milk fat-globule membrane. J. Am. Oil Chemists' Soc. 41:139.

Wooley, J. G. and W. H. Sebrell. 1945. Niacin (nicotinic acid), an essential growth factor for rabbits fed a purified diet. J. Nutr. 29:191.

Young, C. G. 1961. Determination of the glyceride structure of fats. J. Am. Oil Chemists' Soc. 38:62.

APPENDIX

Appendix A. Lipid content of rat feces.

Rat No.	Percent lipids	Percent neutral lipids
A 1	2.38	31.8
A 2	3.32	32.2
A 3	3.65	31.3
B 1	13.11	27.3
B 2	15.26	19.7
B 4	10.03	26.5
C 2	9.36	34.4
C 3	15.68	36.9
C 4	10.97	30.7

Appendix B. Fatty acid composition of triglycerides isolated from rat feces.

Rat No.	Fatty acid composition								
	12:0	14:0	16:0	16:1	18:0	18:1	18:2	18:3	20:4
A 1	1.3	61.3	13.9	4.7	1.0	14.1	3.5	T	T
A 2[1]	T	52.2	13.3	5.6	T	17.3	6.2	T	0
A 3[2]	0	79.3	9.8	6.7	1.3	0	2.0	0	0
B 1[3]	0	43.9	18.4	5.9	5.9	14.6	0.4	T	0
B 2	0	41.9	15.3	4.5	0.8	26.6	8.9	T	0
B 4[4]	0.1	53.1	13.3	6.9	1.0	14.8	8.2	T	2.4
C 2	0	52.7	19.7	4.9	T	22.7	T	T	0
C 3[5]	0	53.4	14.8	2.2	2.0	17.5	9.0	0.9	0
C 4	T	42.7	19.3	7.5	1.9	18.4	4.2	0.2	0

[1]*1.7 percent 20:2 and 3.2 percent 20:3 were detected.
[2] 0.7 percent 20:3 was detected.
[3] Trace 20:3 was detected.
[4] 0.1 percent 10:1 was detected.
[5] 0.1 percent 20:3 was detected.
* Tentative identification.

Appendix C. Fatty acid composition of diglycerides isolated from rat feces.

	Fatty acid composition								
Rat No.	12:0	14:0	16:0	16:1	18:0	18:1	18:2	18:3	20:4
A 1	T	71.4	10.2	5.4	1.5	7.3	4.3	T	T
A 2[1]	T	73.5	4.8	3.3	T	1.4	2.3	0	4.9
A 3	0	81.1	9.8	2.9	0.5	3.6	1.3	0.4	0.2
B 1	0	64.9	14.9	6.4	3.5	9.2	1.0	T	0.2
B 2[2]	0	66.0	11.1	4.6	1.1	10.4	4.3	0.8	0
B 4	1.7	64.3	11.4	5.1	T	6.4	2.6	T	4.7
C 2[3]	0	78.3	12.0	2.0	1.3	5.5	0.9	T	0
C 3[4]	0	56.4	12.4	2.0	0.8	9.7	5.9	T	0
C 4	0	62.8	14.8	3.9	1.3	6.0	1.8	T	9.6

[1]* 5.6 percent 20:2 was detected.
[2] 1.6 percent 20:2.
[3] Trace of 22:1 was detected.
[4] 5.6 percent 22:1 was detected.
* Tentative identification.

Appendix D. Fatty acid composition of monoglycerides-free fatty acids isolated from the feces of rats.

	Fatty acid composition								
Rat No.	12:0	14:0	16:0	16:1	18:0	18:1	18:2	18:3	20:4
A 1	T	77.2	7.4	4.8	0.4	6.8	3.1	T	T
A 2[1]	0	85.8	4.9	3.8	T	1.1	1.1	T	1.9
A 3	0	58.7	24.1	5.0	T	8.7	0.4	0	0
B 1	0	90.2	7.6	1.7	T	T	T	T	0
B 2	0	72.8	11.4	2.7	1.4	7.6	3.8	T	0
B 4	0	64.3	13.6	3.2	1.1	10.3	6.2	1.3	0
C 2	0	76.9	9.1	4.4	1.9	5.8	1.8	T	0
C 3[2]	0	65.6	5.8	8.5	0.9	4.5	3.1	0	17.7
C 4[3]	0.4	14.5	4.5	1.3	0.4	1.7	0.7	T	51.1

[1]* 1.3 percent and 1.7 percent 17:0 and 20:2 were detected.
[2] Trace 22:1 was detected.
[3] 21.1 percent, 4.0 percent and 0.3 percent 22:1, 22:2 and 22:3 were detected.
* Tentative identification.

Appendix E. Fatty acid composition of rat subcutaneous fat.

	Fatty acid composition								
Rat No.	12:0	14:0	14:1	16:0	16:1	18:0	18:1	18:2	18:3
A 1	0	0.9	0.2	15.9	6.9	2.4	35.0	33.7	5.1
A 2	T	1.0	0.2	16.6	4.4	2.5	36.3	36.5	2.4
A 3	T	1.7	0.8	14.1	6.4	3.6	33.1	33.2	7.2
A 4*	0	1.1	0.4	14.2	6.7	3.3	35.4	33.6	5.3
B 1	T	1.1	0.4	16.2	6.2	4.4	32.6	33.2	5.9
B 2	T	0.8	0.3	14.8	8.5	2.2	37.2	32.9	3.1
B 3	0	1.1	0	16.5	11.4	1.2	32.8	34.2	2.3
B 4	T	1.2	0.2	17.0	5.8	4.0	35.3	32.3	3.2
C 1	T	3.7	0.5	23.3	5.9	19.1	31.1	13.7	2.1
C 2	0	1.0	0.3	14.4	7.2	4.1	35.4	33.4	4.0
C 3	T	0.8	0.2	16.3	5.4	2.8	35.0	36.5	3.1
C 4	T	0.9	0.2	18.4	5.4	3.9	34.3	33.7	3.2

*Tentatively identified.

Appendix F. Fatty acid composition of rat perirenal fat.

	Fatty acid composition									
Rat No.	12:0	14:0	14:1	16:0	16:1	18:0	18:1	18:2	18:3	20:4
A 1	0	0.8	0.2	16.5	3.4	2.9	36.5	37.2	2.5	0
A 2	T	0.9	0.2	15.8	3.8	3.1	36.3	37.0	2.9	0
A 3	T	1.2	0.5	13.4	7.1	4.4	32.1	31.6	9.5	0
A 4	0	0.7	0.2	13.4	6.3	3.2	33.4	32.9	4.7	0
B 1	T	0.9	0.2	16.7	4.7	3.9	33.5	35.2	4.7	0
B 2	T	0.7	0.1	17.6	4.4	2.8	37.9	34.3	2.1	0
B 3	0	0.8	0.3	17.4	5.6	2.9	34.3	35.2	3.5	0
B 4	T	1.0	0.2	16.1	6.6	5.4	30.7	32.5	7.7	0
C 1	0.1	1.4	0.4	15.7	8.4	7.8	31.3	29.3	5.6	0
C 2	0	0.9	0.2	13.9	2.9	3.5	36.2	39.1	3.3	0
C3	0	0.7	0.2	16.0	2.9	3.7	34.2	37.2	3.5	0
C 4	T	0.8	0.3	18.1	4.7	3.7	33.8	35.0	3.6	0

*Tentative identification.

Appendix G. Liver analyses

Rat No.	Liver weight g	Liver percent body weight	Liver percent total lipids	Percent neutral lipids
A 1	12.619	2.53	5.67	23.91
A 2	17.905	3.57	3.67	17.96
A 3	17.751	3.32	3.46	15.81
A 4	9.896	2.17	4.64	19.87
B 1	11.816	2.29	5.92	23.57
B 2	16.301	2.89	6.22	28.95
B 3	28.380	4.37	3.32	42.24
B 4	13.741	2.87	7.35	14.27
C 1	20.796	6.49	3.39	25.36
C 2	13.722	2.66	6.94	52.00
C 3	12.958	2.51	5.22	40.23
C 4	20.381	3.25	5.66	20.85

Appendix H. Fatty acid composition of triglycerides isolated from rat liver.

	Fatty acid composition							
Rat No.	12:0	14:0	16:0	16:1	18:0	18:1	18:2	18:3
A 1	T	3.4	28.6	2.8	2.5	32.3	29.0	0.1
A 2	T	10.8	30.5	2.9	1.9	28.1	25.4	0.5
A 3[1]	0.1	9.8	26.5	5.3	4.1	25.3	28.0	1.0
A 4	0.1	3.7	22.1	4.5	2.5	34.6	30.7	1.3
B 1	T	3.0	25.1	3.6	4.0	30.9	32.4	0.9
B 2	T	2.0	26.5	3.4	2.3	33.4	31.0	1.1
B 3	T	2.7	28.2	3.2	3.1	35.7	27.7	0.5
B 4	T	9.5	24.9	3.1	3.1	34.1	27.6	0.8
C 1	0	2.3	32.9	4.3	4.2	25.2	31.0	0.1
C 2	0	13.2	24.1	2.4	6.5	36.4	17.4	T
C 3	0	3.4	25.4	4.4	4.6	23.7	37.7	0.9
C 4	0	1.8	25.9	3.6	3.0	27.7	37.1	1.0

[1]* Traces of 8:0 and 10:0 were detected.
* Tentative identification.

Appendix I. Fatty acid composition of cholesterol esters isolated from rat liver.

	Fatty acid composition							
Rat No.	12:0	14:0	16:0	16:1	18:0	18:1	18:2	18:3
A 1	0.2	36.8	28.2	2.3	4.9	15.8	11.9	T
A 2[1]	1.0	54.5	21.4	2.5	1.5	7.8	5.4	0
A 3[2]	2.3	41.7	16.5	1.6	4.0	6.8	7.1	0
A 4[3]	0.8	37.1	18.5	17.3	3.2	11.0	9.0	0.2
B 1	0	13.9	33.5	2.5	9.6	24.5	16.1	T
B 2	0.4	50.7	26.4	4.6	3.2	8.7	6.1	0
B 3	0.3	33.7	26.2	3.1	2.1	20.4	13.2	0.9
B 4	0.5	55.9	18.1	0.5	4.5	13.9	6.7	0
C 1	3.3	6.8	53.3	0.2	7.7	12.9	14.7	0.5
C 2	0	63.7	15.2	0.2	4.7	12.1	4.0	0
C 3	0	45.6	24.6	0	4.3	14.7	10.1	0
C 4	0	38.5	20.7	2.5	5.5	17.3	15.7	0

[1]* 0.6, 2.2 and 2.9 percent 8:0, 10:0, and 14:1 were detected.
[2] 18.5 percent 8:0 and 10:0 and 1.5 percent 14:1 were detected.
[3] Trace, 1.7 and 0.9 percent 10:0, 14:1, 15:0 were detected.
* Tentative identification.

Appendix J. Fatty acid composition of diglycerides isolated from rat liver.

	Fatty acid composition							
Rat No.	12:0	14:0	16:0	16:1	18:0	18:1	18:2	18:3
A 1[1]	0.5	27.4	32.0	4.0	3.5	14.9	15.1	0.7
A 2[2]	0.6	43.1	27.0	2.3	1.2	11.1	12.7	1.3
A 3[3]	0.4	41.8	25.2	2.7	2.7	9.2	8.5	0
A 4	0.5	48.1	19.3	7.8	2.2	11.5	10.0	0.7
B 1	0	40.9	23.3	2.3	3.3	16.0	13.2	T
B 2	T	44.8	17.2	1.3	2.3	19.6	14.4	0.4
B 3	T	16.9	27.4	3.2	1.3	23.2	27.2	0.8
B 4[4]	0.3	54.9	17.2	2.7	5.3	11.6	8.3	T
C 1	0	33.2	47.1	0.1	4.8	4.2	7.7	3.0
C 2	0	68.0	14.5	0.5	3.9	9.9	3.0	0
C 3	0	34.8	21.3	1.0	6.6	16.8	19.5	T
C 4	0	36.2	21.4	2.5	6.9	14.2	18.4	0.6

[1]* 1.8 percent 14:1 was detected.
[2] Trace of 10:0 was detected.
[3] 9.6 percent 17:0 was detected.
[4] Trace of 10:0 was detected.
* Tentative identification.

Appendix K. Fatty acid composition of monoglyceride-free fatty acid fraction isolated from rat liver.

	Fatty acid composition							
Rat No.	12:0	14:0	16:0	16:1	18:0	18:1	18:2	18:3
A 1	0	33.6	33.3	4.1	4.9	10.4	13.2	0
A 2	0.1	12.0	37.0	3.6	4.3	21.7	20.4	0.9
A 3	0.6	40.6	30.7	4.4	7.0	8.5	8.2	0
A 4	0.3	59.5	17.6	6.7	2.1	6.9	7.0	0
B 1[1]	0	39.4	28.4	0.9	6.8	13.6	9.5	0
B 2	0.2	35.3	23.5	2.7	5.4	16.8	15.8	0.4
B 3	T	33.8	20.3	3.6	3.8	18.6	19.8	0.1
B 4	0.1	27.2	31.3	2.7	7.0	15.0	16.0	0.8
C 1	10.6	20.5	32.4	0	1.3	18.6	16.5	0
C 2	0	7.1	33.6	0	23.0	19.3	17.1	T
C 3	0	4.5	18.4	0	35.9	18.3	22.5	T
C 4	0	22.0	29.7	3.0	11.0	15.5	18.4	0.6

[1]* 2.1 percent 17:0 was detected.
* tentative identification.

Appendix L. Fatty acid composition of phospholipids isolated from rat liver.

	Fatty acid composition								
Rat No.	12:0	14:0	16:0	16:1	18:0	18:1	18:2	18:3	20:4
A 1[1]	0.2	0.6	30.4	0	29.2	12.9	13.4	T	13.3
A 2[2]	T	1.5	23.6	0	26.9	12.9	16.7	T	18.2
A 3[3]	T	1.2	26.4	0	28.1	15.3	13.6	T	15.4
A 4[4]	T	0.8	30.0	0	26.3	14.7	14.3	T	14.0
B 1[5]	T	1.2	23.8	0	36.0	11.3	9.0	T	19.0
B 2[6]	T	0.5	27.7	0	31.0	10.0	11.5	T	18.9
B 3[7]	T	0.5	23.7	0	30.7	14.2	12.3	T	18.0
B 4[8]	T	0.7	24.5	0	32.6	10.7	11.6	T	19.6
C 1[9]	T	1.4	26.0	0	27.2	11.3	18.8	T	15.3
C 2[10]	T	4.0	30.4	0	32.8	11.8	13.1	T	6.6
C 3[11]	T	0.3	24.7	0	36.6	12.3	14.2	T	11.7
C 4[12]	T	0.5	21.8	0	31.8	12.6	17.4	T	15.8

[1]*Trace of 14:1 was detected.
[2] Trace of 14:1 was detected.
[3] Trace of 14:1 was detected.
[4] Trace of 14:1 was detected.
[5] Trace of 14:1 was detected.
[6] Trace of 14:1 was detected.
[7] Trace of 14:1 was detected.
[8] Trace of 14:1 was detected.
[9]Trace of 10:0 was detected.
[10]1.1 percent 10:0 was detected.
[11]Trace of 10:0 was detected.
[12]Trace of 10:0 was detected.
* Tentative identification.

Appendix M. Blood serum lipids

Rat No.	ml. serum	mg total lipids	mg/ml	Total neutral lipids	Percent neutral lipids	Percent phospho-lipids
A 1	4.0	39.7	9.93	24.7	62.22	37.78
A 2	3.5	31.5	9.00	16.9	53.65	46.35
A 3	3.1	25.7	8.29	13.1	50.97	49.03
B 1	4.1	170.8	41.66	14.0	8.20	91.80
B 2	4.7	61.8	13.15	10.7	17.31	82.69
B 4	4.1	133.8	32.56	7.6	5.69	94.31
C 2	4.2	118.7	28.26	14.2	11.96	88.04
C 3	4.3	139.3	32.40	11.2	8.04	91.96
C 4	1.3	18.4	14.15	7.2	3.91	96.09

Appendix N. Fatty acid composition of triglycerides isolated from rat blood serum.

	Fatty acid composition							
Rat No.	12:0	14:0	16:0	16:1	18:0	18:1	18:2	18:3
A 1[1]	0.2	7.8	29.6	6.2	12.5	39.3	3.7	T
A 2[2]	0.5	20.8	24.5	4.5	8.8	30.6	8.4	0.5
A 3[3]	0.7	35.5	19.9	4.9	5.5	25.1	7.7	0.7
B 1[4]	0.5	19.0	28.4	5.1	8.1	24.5	13.4	0.5
B 2	0.6	81.7	6.9	4.1	1.9	3.2	1.6	0
B 4	0.9	61.2	11.3	3.0	4.9	11.2	3.8	3.6
C 2[5]	4.4	56.6	9.9	1.9	2.6	7.9	5.7	0
C 3[6]	0.3	35.7	12.8	3.0	4.2	14.2	11.6	0
C 4[7]	1.3	58.3	11.9	2.4	4.6	12.7	6.6	1.4

1* 0.7 percent 14:1 was detected.
2 0.8 and 0.6 percent 10:0 and 14:1 were detected.
3 Trace of 10:0 was detected.
4 Trace of 10:0 was detected.
5 10.4 percent 10:0 and 0.5 percent 11:0 were detected.
6 18.2 percent 10:0 was detected.
7 Trace 10:0 and 0.8 percent 14:2 were detected.
*Tentative identification.

Appendix 0. Fatty acid composition of cholesterol esters isolated from rat blood serum.

	Fatty acid composition							
Rat No.	12:0	14:0	16:0	16:1	18:0	18:1	18:2	18:3
A 1[1]	1.5	71.5	10.1	5.8	1.3	6.4	1.5	0
A 2[2]	0.5	50.9	14.0	2.5	2.7	11.5	14.0	0
A 3	1.0	73.2	9.6	1.9	1.9	4.5	8.0	0
B 1[3]	1.3	77.8	6.1	3.3	3.0	2.4	2.8	3.2
B 2	1.3	81.7	7.2	1.0	2.5	3.5	2.8	0
B 4[4]	3.5	77.4	6.5	0.8	1.3	2.2	1.6	0
C 2[5]	1.1	79.5	6.2	1.7	1.4	2.6	3.3	T
C 3	0.5	44.5	18.8	2.0	3.5	14.4	11.8	0
C 4[6]	2.1	68.3	11.9	1.7	5.0	6.1	4.8	0

[1]* 1.9 percent 14:1 was detected.
[2] 3.9 percent 14:1 was detected.
[3] Trace of 10:0 was detected.
[4] 2.6 percent 10:0 and 3.5 percent 14:1 were detected.
[5] 4.2 percent 14:1 was detected.
[6] Traces of 8:0 and 10:0 were detected.
* Tentative identification.

Appendix P. Fatty acid composition of diglycerides isolated from rat blood serum.

	Fatty acid composition							
Rat No.	12:0	14:0	16:0	16:1	18:0	18:1	18:2	18:3
A 1[1]	0.4	58.6	16.6	2.4	5.1	13.5	2.3	0
A 2[2]	0.3	25.0	32.5	3.6	10.9	20.0	4.7	0
A 3	1.0	76.0	11.3	1.9	1.5	5.4	8.0	0
B 1[3]	1.4	62.3	11.9	2.1	7.1	9.6	4.1	0.9
B 2	0.5	53.4	15.4	2.6	3.2	14.8	4.8	5.3
B 4[4]	0.8	64.4	14.8	4.1	3.3	10.4	2.3	0
C 2[5]	18.4	38.6	11.8	1.3	0.9	6.0	10.6	0
C 3	1.1	79.8	7.7	1.5	1.9	3.2	4.4	0
C 4	0	62.3	7.6	3.4	8.4	10.2	8.0	0

[1]* 1.0 percent 14:1 was detected.
[2] Trace of 10:0 was detected.
[3] 2.8 percent 17:0 was detected.
[4] Trace of 10:0 was detected.
[5] 12.3 percent 10:0 was detected.
* Tentative identification.

Appendix Q. Fatty acid composition of monoglycerides-free fatty acids isolated from rat blood serum.

	Fatty acid composition							
Rat No.	12:0	14:0	16:0	16:1	18:0	18:1	18:2	18:3
A 1[1]	0.5	22.4	36.3	5.9	12.5	19.8	1.5	0
A 2[2]	0.7	37.9	27.6	3.8	10.3	14.6	3.2	0.3
A 3	1.2	42.0	23.5	4.6	6.9	16.1	5.6	0
B 1	0.3	13.0	40.1	4.4	15.3	20.7	6.5	0
B 2	0.6	60.1	17.9	3.2	3.4	11.6	3.2	0
B 4[3]	0.8	55.6	19.6	3.5	4.3	11.3	2.0	1.2
C 2[4]	0.3	86.0	7.3	1.1	0.7	2.0	2.6	0
C 3	0.7	59.9	21.5	2.2	3.7	7.5	4.4	0
C 4	1.0	34.3	26.1	2.0	7.2	18.1	10.4	1.0

[1]* 0.8 percent 16:2 was detected.
[2] 1.6 percent 16:2 was detected.
[3] unidentified 1.5 percent between 14:0 and 16:0.
[4] Trace of 10:0 was detected.
* Tentative identification.

Appendix R. Fatty acid composition of phospholipids isolated from rat blood serum.

	Fatty acid composition								
Rat No.	12:0	14:0	16:0	16:1	18:0	18:1	18:2	18:3	20:4
A 1[1]	0.8	9.8	31.6	22.1	18.5	11.1	0.1	0	4.4
A 2[2]	1.1	10.7	37.2	0	30.1	12.6	8.3	0	0
A 3[3]	T	3.6	37.0	0	29.0	16.8	10.9	T	2.7
B 1[4]	0.3	4.0	30.4	0	28.7	14.1	15.3	T	7.3
B 2[5]	1.1	11.2	26.3	0	29.8	15.6	12.5	T	2.9
B 4[6]	0.3	6.6	28.4	0	32.8	15.7	14.7	T	1.4
C 2	0.3	9.3	23.5	0	27.5	11.1	16.7	T	11.2
C 3[7]	0.7	20.6	21.1	0	26.7	9.8	14.1	T	6.9
C 4[8]	2.6	18.9	20.0	1.5	27.9	13.5	12.1	T	3.6

[1]*Trace of 10:0 and 1.6 percent 17:0 were detected.
[2] Trace of 10:0 and 17:0 were detected.
[3] Trace of 10:0 was detected.
[4] Trace of 20:3 was detected.
[5] Traces of 8:0, 10:0, 20:3, and 1.3 percent 17:0 were detected.
[6] Trace of 20:3 was detected.
[7] Trace of 10:0 was detected.
[8] Trace of 10:0 was detected.
*Tentative identification.

Appendix S. Composition of basal and pretest diets.

Basal diet

Ingredient	Percent
Vitamin free casein	21.10
Alphacel cellulose	16.45
Sucrose	58.45
Salt mixture	4.00
Vitamin mixture	336.275 g/100 lb

Pretest diet

Ground shelled corn	46.100
Sucrose	5.000
Soybean oil meal	20.000
Fishmeal	10.000
Alfalfa meal	5.000
Dried skimmilk	10.000
Corn oil	3.000
Super trace mineral salt	0.500
B-vitamin supplement	0.125
Vitamin A and D concentrate	.050