THE EFFECT OF VARYING LEVELS OF DIETARY LINOLEATE ON THE FATTY ACID COMPOSITION OF RAT TISSUE PHOSPHOLIPIDS

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ABSTRACT

THE EFFECT OF VARYING LEVELS OF DIETARY LINOLEATE ON THE FATTY ACID COMPOSITION OF RAT TISSUE PHOSPHOLIPIDS

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Ricardo R. del Rosario

The growth rate of rats raised on a basal diet containing different levels of linoleic acid was followed during the feeding trials. The weight gains were proportional to the amount of linoleic acid in the diet. The animals were killed after the feeding trial and the livers, hearts and blood were recovered. The lipids were extracted from the tissues. The proportions of the different phospholipids in the total lipid extract, when determined by phosphorous analysis, did not reveal any variation with the diet.

The fatty acid compositions of several phospholipids were determined and found to vary with the dietary fat. The major phospholipids, phosphatidylethanolamine (PE) and phosphatidylcholine (PC), exhibited the differences in fatty acid composition observed in total lipid extracts. Both phospholipid classes from rats raised on an EFA deficient diet showed high levels of oleic acid

and eicosatrienoic acid and low amounts of arachidonic and linoleic acids. With higher levels of dietary linoleate, the amounts of oleic and eicosatrienoic acids became low while those of the linoleic acid family were elevated. The saturated fatty acids remained generally unaffected by the dietary fat.

Among the minor phospholipids, cardiolipin presented a unique composition. It accumulated considerable amounts of linoleic acid in animals fed the diet containing corn oil. In the EFA deficient rats, cardiolipin had a reduced level of linoleic acid which was compensated for by an increase in oleic acid content.

The other minor phospholipids, phosphatidylinositol (PI) and phosphatidylserine (PS), exhibited differences not only in the unsaturated fatty acids like the major phospholipids, but also in the saturated fatty acid content.

Sphingomyelin from the liver showed some differences in the fatty acid composition with regard to the diet, but not the sphingomyelin from the heart.

Plasmalogens varied mainly in fatty acid composition with respect to differences in the dietary fat. The plasmalogen content or the aldehyde of either PE or plasmalogen remained unvaried with the diet. Liver PE showed higher levels of plasmalogen than PC while in the heart there was more plasmalogen in the PC than in the PE.

Comparison between tissues showed that the heart lipids contained more plasmalogen than the liver lipids.

Analysis of the fatty acid distribution in the phospholipid molecules indicated that the variation in the fatty acid composition occurred mostly in the β-position.

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INTRODUCTION

The well-being of an individual depends in part on the quality of the food he eats. The lack or excess of a given nutrient may lead to a diseased condition which if not cured may finally lead to death. This is true of food nutrients, such as vitamins, amino acids and minerals which are considered essential to animals.

Of special interest are the fatty acids which have been implicated in the genesis of cardiovascular diseases particularly atherosclerosis which is a major health problem in some countries. Cardiovascular diseases are most important not only because they rank as a number one killer but also because many middle-aged people are affected. In the United States alone, more than 100,000 deaths have been attributed to this disease of which 25,000 were persons under 65 years of age.

Many studies have been conducted to relate dietary fat to cardiovascular diseases, but whether or not there is a relationship between the two is still to be established. Mohrhauer and Holman (1963) reported a definite relationship between dietary fats and tissue fatty acid composition of rats. This was especially true of the phospholipids which appeared to be highly affected by the

dietary fat. It has been shown to affect blood cholesterol level, a popularly believed causal factor in the development of cardiovascular diseases. It has also been known to affect the activity of certain enzyme systems (Hayashida and Portman, 1963).

In view of the importance of the essential fatty acids in the diet and the body, it was proposed to study the effects of levels of linoleic acid in the diet on the fatty acid composition of different classes of phospholipids, to determine the site of major compositional differences in different tissues of rats and to determine the composition and extent of participation of plasmalogen on the location of the products of dietary linoleate in rats.

REVIEW OF LITERATURE

Historical Background

The requirement of many animal species for certain substances for normal growth and development has been discovered to include vitamins, minerals, amino acids and fats. Among these substances, fats have recently assumed great importance because of an apparent relation to cardiovascular diseases. The first report indicating the possible special requirement of animals for fat was made by Evans and Burr (1928). These workers found that rats fed a purified diet developed syndromes which had not been described previously. The rats developed necrosis of the tail, scaly skin, loss of weight, kidney degeneration and increased water consumption. Burr and Burr (1929) later confirmed these results by raising rats on a fat free diet until they developed the symptoms. They showed that addition of olive oil, lard, corn oil, poppy seed oil or linseed oil resulted in resumption of growth and disappearance of symptoms. Further studies (Burr and Burr, 1930) revealed that the curative effect of fat was due to the fatty acids and not the glycerol moiety. Furthermore, it was reported that saturated fatty acids like lauric,

palmitic and stearic acids did not possess curing power. Unsaturated fatty acids, particularly linoleic acid, were effective in alleviating the symptoms but oleic acid had a doubtful curing effect. They also observed that complex unsaturated oils like corn oil or cod-liver oil were more effective than single fatty acids in eliminating essential fatty acid deficiency symptoms.

Essential Fatty Acid Deficiency Symptoms

In addition to the original deficiency syndromes described by Burr and Burr (1929), other manifestations which were associated with essential fatty acid deficiency in rats have been described by later workers. Leduc and Wilson (1964) isolated mitochondria from essential fatty acid (EFA) deficient and normal rat liver and showed that the mitochondria from the deficient rats were more enlarged and spherical compared to the normal mitochondria.

Wesson and Burr (1931) studied the metabolism of EFA deficient rats and reported that a high metabolic rate was observed at the onset of deficiency symptoms. The basal respiratory quotient and body temperature were normal. Feeding EFA deficient rats with a carbohydrate diet gave a respiratory quotient greater than one, indicating the synthesis of fat from carbohydrate. Further studies (Levin et al. 1957); Smith and DeLuca, 1963) showed that mitochondria isolated from liver of EFA

deficient rats oxidized Krebs cycle acids faster than the control, resulting in larger oxygen consumption. This effect was attributed to uncoupling of oxidative phosphorylation (Klein and Johnson, 1954; Tulpule and Williams, 1955; Levin et al., 1957). Levin and his co-workers (1957) suggested that the uncoupling of oxidative phosphorylation was due to the susceptibility to damage of EFA deficient mitochondria. This suggestion was partially supported by Hayashida and Portman (1963) who reported a high degree of susceptibility to swelling of EFA deficient mitochondria. Smith and DeLuca (1964) further suggested that the changes were partially related to changes in mitochondrial structure chiefly due to enlargement (Leduc and Wilson, 1964).

Caster and Ahn (1963) even reported the appearance of notching in rat electrocardiograms which could be eliminated by feeding with essential fatty acids.

Since phospholipids are major constituents of membranes, both structural and functional changes have been explained in terms of alterations in the fatty acid composition. De Pury and Collins (1963) studied the acetylcholine stimulation of P^{32} uptake in phosphatidic acid which would indicate a disruption of microsomal membrane. They found that there was an increase in the uptake of P^{32} in phosphatidic acid which would indicate disruption during deficiency. MacMillan and Sinclair (1958) and

Kramer and Levine (1953) reported on the increased permeability to water of the skins of EFA deficient rats.

Thacker (1956) and Ahluwalia et al. (1967) associated EFA deficiency in rabbits with diminished growth, low feed efficiency and loss of hairs. It was also shown (Ahluwalia et al. 1965) that rabbits fed with EFA deficient diets exhibited extensive degeneration of the semeniferous tubules.

Dogs raised on a low fat diet were shown by Hansen and Weise (1943) to develop dry skin and coarse hair after the first three months of feeding. Thereafter, alterations in the skin led to the formation of flaky desquamations with larger scales and scurfy specks all over the body. They later suggested (Hansen et al. 1954) that EFA was necessary for the maturation of epithelial and sebaceous cells.

Rieser (1950) raised chicks on a fat-free diet for weeks and claimed that the growth rate was slow and that a edematous subcutaneous layer having the appearance of jelly developed. After the fourth week, death resulted from the deficiency. In the case of layers raised on diets containing low amounts of EFA, egg production dropped. Fertility and hatchability of the eggs became proportional to the amount of linoleate in the diet above 10 mg/hen/day.

Boyd and Edwards (1966) found that EFA deficient chicks were highly susceptible to E.coli infection and to the development of a respiratory disease syndrome characterized by bronchial exudate. Both symptoms could be prevented by the incorporation of soybean or corn oil in the diet.

Witz and Beeson (1951) associated the formation of skin lesions in swine with EFA deficiency which was confirmed by Sewell and Miller (1966). Other workers (Hill et al. 1957, 1961; Sewell and McDowel, 1966) could not reproduce the above claims or poor weight gains.

The first demonstration of EFA deficiency in fish was reported by Nicolaides and Woodall (1962). They raised chinook salmon on an artificial diet and found that those fish fed a linoleate-free diet did not produce the same degree of pigmentation as fish fed a control diet. This was also found to be true in trout (Higashi et al. 1966). Lee and his co-workers (1969) claimed that high mortality and slow growth rates were also exhibited by EFA deficiency in trout.

Influence of Sex and Age on the Response to EFA

The influence of sex on the response to EFA has been observed with growth rate studies. Burr and Burr (1929, 1930) raised rats on a fat-free diet and found that

male rats generally attained only 70% of the weight of the control compared to the female rats which averaged 80%. They also reported that the EFA deficient males did not mate, while the female rats would mate during ovulation.

Greenberg and his co-workers (1950) studied the EFA requirement of rats using growth studies and reported that females required 10-20 mg/day and about 100 mg/day was required by the males. More recent studies by Mohrhauer and Holman (1963) established a requirement of 1% EFA of the calories for male rats based on tissue analysis. This study also showed the relationship of EFA requirement to the calorie intake. Later work by Pudelkewics et al. (1968) reported a modified requirement of 1.3% linoleate as calories for the male and 0.5% for the female.

Tissue analyses have also shown (Lyman et al., 1967) that female rats raised on a EFA diet maintained higher levels of arachidonic acid and stearic acid in the plasma phospholipid and cholesterol esters than the males.

Like sex, age affects the utilization of EFA in the body. Barki and his co-workers (1947) reported on the development of EFA deficiency symptoms in adult rats. The rats were restricted in calorie intake until the weight dropped to about 1/2 the original weight and then were shifted to a fat-free diet. After a number of weeks, the rats developed typical EFA deficiency symptoms which spontaneously disappeared on prolonged feeding of a EFA

deficient diet. Aaes-Jorgensen et al. (1958) reported a similar finding for rats raised on a EFA-free diet or on a low fat diet (1%) for 35 weeks.

In mice, (Decker et al., 1950) feeding with a EFA-free diet resulted in a chronic state of deficiency where none of the external symptoms characteristic of acute EFA deficiency were visible.

Analysis of the tissues of adult rats raised on a fat-free diet (Barki et al., 1949) showed that the concentration of dienes and tetraenes were low at the stage when symptoms of EFA deficiency were present.

Development of symptoms of EFA deficiency is difficult in the adult stage of hogs and cows. It is possible to produce the external symptoms when feeding is started with either weanling pigs (Witz and Beeson, 1951; Hill et al., 1957) or calves (Lambert et al., 1958). Cows raised on a EFA-free diet showed depressed iodine value in blood lipids compared to that of normal control animals (Gibson and Hoffman, 1939).

Metabolism of Polyunsaturated Fatty Acids (PUFA)

It has long been recognized that certain unsaturated fatty acids in the diet give rise to particular PUFA in the body lipid. This was concluded from feeding single fatty acids to animals and determining the different tissue fatty acids after the feeding trials.

Reiser (1951) suggested that fragments from ingested fatty acid containing double bonds might combine with dietary fatty acid to form the more highly unsaturated fatty acids.

Mead and his co-workers (1953) injected rats with carboxyl-labelled acetate and analyzed the lipid from the organs and adipose tissue. They reported that the activity was mostly found in the carboxyl group of arachidonic acid and little or no activity was present in carbon 18. Later Howton et al. (1954) prepared carboxyl-labelled linoleic acid and fed it to rats. They isolated arachidonic acid from some organs and the adipose tissue and reported a high activity in the carbon 1 and 3 with little or no activity in carbons 4 to 20 indicating the direct incorporation of linoleic acid into arachidonic acid.

Thomasson (1953) isolated γ-linolenic acid from the seeds of Oenothera lamarckiana and labelled the carboxyl group with C^{14}. He fed them to rats and found that 90% of the activity were recovered in arachidonic acid.

Howton and Mead (1960) later using a synthetic homo-γ-linolenic acid (8,11,14 eicosatrienoic acid) reported its direct conversion to arachidonic acid in rat tissues.

These findings allowed Mead (1961) to formulate the pathway for the conversion of linoleic acid to arachidonic acid as follows:

18:2 ω 6 → 18:3 ω 6 → 20:3 ω 6 → 20:4 ω 6

Klenk and Mohrhauer (1960) using eicosa-11,14 dienoic acid found that this acid could also be transformed into arachidonic acid in rats indicating the existence of an alternate pathway:

18:2 ω 6 → 20:2 ω 6 → 20:3 ω 6 → 20:4 ω 6

This finding also showed that linoleic acid could undergo either elongation or dehydrogenation in its conversion to arachidonic acid.

Several groups of workers (Nugteren, 1962; Stoffel, 1963; Stoffel and Ach, 1964) later developed an in vitro synthesizing system capable of both dehydrogenation and elongation using rat liver microsomes.

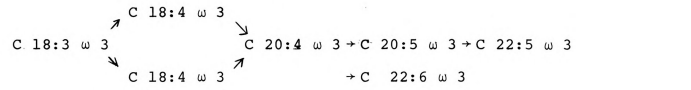
Later Brenner and his co-workers (1968) were able to separate the two systems. Marcel et al. (1968) showed that the path to eicosa-8,11,14 trienoic acid via γ-linolenic acid was favored.

18:2 ω 6 → 18:3 ω 6 → 20:3 ω 6 → 20:4 ω 6

Using the same technique in elucidating the metabolic pathways, Kayama et al. (1963) studied the transformation of carboxyl-labelled linolenic acid in fish. They reported that in kelp bass the major fatty acid products from linolenic acid were 20:5 and 22:6 with most of the activity in carbon 18.

Klenk and Mohrhauer (1960) synthesized several possible intermediates and followed their transformation

in rats by isolation and degradation of the labelled PUFA which lead to the following scheme:



Mead and Slaton (1956) in trying to isolate the intermediates in linoleate metabolism, found that the trienoate in animals in a EFA deficient state is not linolenic acid but a 5,8,11 eicosatrienoic acid. This was later shown by Fulco and Mead (1959) to be derived from oleic acid.

Effect of EFA on the Tissue Lipid Composition

In order to understand the role of EFA in the body, it is necessary to determine its fate and location in different tissues. Early workers quickly recognized the effect of dietary EFA on tissue composition. Sinclair (1930, 1931) reported, in a series of papers, the changes in the tissues of animals raised on a fat-free diet. He revealed that the iodine number of the lipid fraction in rat tissues was low during EFA deficiency. He also noted that the depot fat in rats may have lower or higher iodine numbers depending upon the kind of fat used in the diet. In addition, he recognized the tendency of phospholipids to contain highly unsaturated fatty acids.

The decrease in unsaturation under a EFA deficient state was shown by Nunn and Smedley-Maclean (1938) to be due to the accumulation of a trienoic acid which he isolated from the rat liver. Klein and Johnson (1954) demonstrated that these changes could be traced to the subcellular granules and found them to occur six weeks before the onset of external symptoms. On the other hand, the increase in iodine number of lipids in tissues of rats on a normal diet was due to the accumulation of certain PUFA containing four, five and six double bonds (Reiser, 1950; Widmer and Holman, 1950). Both workers (Holman, 1951; Reiser, 1951), using different test animals, reported the association of a given dietary fatty acid to a particular fatty acid in the tissue.

The development of gas chromatography made fatty acid analysis definitive especially in terms of chain length and the proportion of double bonds compared with the older spectrophotometric analysis after alkali isomerization. Holman (1960) analyzed the total phospholipids in three rat tissues and found the increasing amount of eicosatrienoic acid and decreasing amounts of both linoleic acid and arachidonic acid to be associated with progressive EFA deficiency. He reported that more pronounced effects occurred in the liver and plasma than in the heart. Aside from the above-mentioned fatty acids, palmitoleic acid and oleic acid also increased (Mead,

1957). The saturated fatty acids did not appear to be affected by the diet. Thus, essential fatty acids were replaced by the non-essential fatty acids which the animal body has the capacity to synthesize.

Similar changes have been observed by Walker (1967) in rat erythrocytes from EFA deficient rats. Under the EFA deficient state, both oleic and eicosatrienoic acids accumulated. These were then replaced by linoleic acid and arachidonic acid when the diet was supplemented with corn oil.

The brain and the nervous system appeared to be highly stable (Korey and Orchen, 1959; Pritchard, 1963; Walker, 1968) as the lipids there appeared unaffected by diets. Gidez (1964) observed similar changes in adrenal glands but only under extreme conditions of EFA deficiency.

Yu and associates (1966) reported that in rat leucocytes and granules low amounts of linoleic acid and arachidonic acid were present during EFA deficiency which in turn was accompanied by elevated amounts of oleic acid and eicosatrienoic acid as well as palmitoleic acid.

Walker (1968) and Sewell and Miller (1966) studied the effect of deficiency of EFA on the testes and reported that arachidonic acid was readily incorporated into the testes and was later followed by the accumulation of docosapentaenoic acid. Eicosatrienoic, oleic and

palmitoleic acids all decreased with corn oil supplementation in the diet.

The variation in the fatty acid incorporation of the different tissues led to speculation of variation between the phospholipid molecules. Harris and Robinson (1960) recognized the heterogeneity of phospholipid with regard to its fatty acid composition. Studying the incorporation of P^{32} into lecithin, he found varying amounts of labeled phosphorous in the different species of lecithin which appeared to be related to the fatty acid composition. He noted that those containing high amounts of arachidonic acid had very low labelling suggesting slow turn-over. He suggested that the presence of arachidonic acid conferred stability upon the molecule. This was confirmed by other workers (Enser and Bartley, 1962) who observed that, even under a EFA deficient state, the intestinal mucosa and muscle of rats were able to maintain their arachidonic acid content.

From the tissue analyses, it became evident that the fatty acid composition of the tissue is a more sensitive indicator for EFA status in the animal than the classical gross deficiency syndromes. Holman (1960) devised a parameter consisting of the ratio of tetraenoic acid and eicosatrienoic acid to indicate the EFA status and also enable the measurement of the minimum requirement for EFA in relation to the calorie intake.

EXPERIMENTAL

Materials

The male weanling rats used for the experiment were obtained from Spartan Research Animal, Haslett, Michigan.

The feed components were purchased from General Biochemicals, Chagrin Falls, Ohio. These consisted of a salt mixture (USP XIV), Vitamin Fortification, casein and α-cellulose. Sucrose was bought from a local store. Corn oil was provided by Durkee Famous Foods, Chicago, Illinois while hydrogenated and non-hydrogenated coconut oil were obtained from the Drew Chemical Corporation.

The methyl esters and phospholipids standards used for gas-liquid and thin-layer chromatographs were obtained from Applied Science Laboratory, State College, Pennsylvania. The Chromosorb W (80/100 mesh sieve) was also obtained from the same source. Silica Gel-G and HR, from Brinkman Instruments, Westbury, New York, were used for all thin-layer chromatographic works. Diethyleneglycol succinate was purchased from Analabs Inc., Hamden, Connecticut. Dimethylacetal standards were prepared from pure aldehydes by methylation with methanolic-HCl.

All solvents were glass distilled. Treatment of the solvents with trichloroacetic acid-2,4-dinitrophenylhydrazine reagent was made when necessary to eliminate aldehydes present. The diethyl ether used for methylation was distilled over potassium hydroxide-ferrous ammonium sulfate to destroy the peroxides.

Snake venom from Crotalus adamanteus, source of phospholipase A, was obtained from Reptile Institute, Spring Harbor, Florida.

Methods

Feeding Trials

Two different groups of Sprague-Dawley weanling rats were used for the feeding experiments. The rats were given feeds that were mixed in the laboratory in order to control the composition in accordance with the formula shown in Table 1 (on page 18).

The dietary fat was varied depending upon the level of linoleic acid required in the treatment. The corresponding fatty acid composition is shown in Tables 2 and 3 (pages 19 and 20).

Based on the linoleic acid content of the dietary fat component, the designated diet would provide the corresponding level of dietary linoleate as percent of the total calories as follows: first feeding trial, diet A,

Table 1.--Composition of basal diet.

Components	Percentage Composition By Weight	By Calorie[1]
Sucrose	58.30	62.3
Casein, vitamin free test	16.67	17.8
Fat[2]	8.25	19.8
Vitamin Mix[3]	3.00	
Salt Mix[4]	0.83	
Non-nutritive fiber (cellulose type)	12.50	

[1]Calories calculated on the basis of 4 cal/gm of carbohydrate and protein and 9 cal/gm of fat.

[2]Coconut oil and corn oil fed separately or as mixtures of the two oils.

[3]Vitamin fortification mixture obtained from General Biochemicals, Chagrin Falls, Ohio.

[4]USP XIV salt mix obtained from General Biochemicals, Chagrin Falls, Ohio.

0.5%; diet B, 6.0%; diet C, 12.0%; second feeding trial, diet D, 0.0%; diet E, 0.5%, diet F, 4.0%; diet G, 12.0%.

The first sixty rats were divided into three equal groups and assigned by random into the treatments. The forty-eight rats used in the second feeding study were divided equally among the four treatments.

The rats were weighed individually and placed one to a cage. Each cage was provided with an automatic

Table 2.--Fatty acid composition[1] of oil mixtures used as dietary fat[2] components for the first feeding trial.

Fatty Acid[3]	Diets		
	A	B	C
6:0	4.1	2.0	0.0
8:0	6.5	3.7	0.0
10:0	6.5	3.7	0.0
12:0	47.9	24.0	0.0
14:0	18.3	9.2	0.0
16:0	8.9	2.4	13.2
18:0	2.2	11.0	3.2
18:1 ω 9	4.7	14.7	24.8
18:2 ω 9	1.0	30.8	60.6

[1]Average fatty acid composition based on the proportion of oils used.

[2]Fat mixtures for the diets were: A - 100% coconut oil; B - 50% corn oil + 50% coconut oil; C - 100% corn oil.

[3]The first number of the fatty acid designation represents the carbon chain length and the number following the colon stands for the number of double bonds. The 'ω' number combination indicates the position of the first double bond from the methyl end of the chain.

watering device and a porcelain dish for the feed. The rats were weighed weekly for the duration of the experiment which lasted five weeks for the first trial and six weeks for the second.

Table 3.--Fatty acid composition[1] of fat mixtures used as dietary fat[2] components for the second feeding trial.

Fatty Acid[3]	Diets			
	D	E	F	G
6:0	5.6	6.3	4.6	0.0
8:0	7.6	7.8	5.4	0.0
10:0	7.3	7.9	5.7	0.0
12:0	49.8	45.9	30.8	0.0
14:0	16.9	18.4	13.3	0.0
16:0	6.6	6.4	8.4	13.2
18:0	6.0	3.9	2.2	3.2
18:1 ω 9	0.0	1.4	10.3	24.8
18:2 ω 6	0.0	2.0	20.2	60.6

[1]Average fatty acid composition based on proportions used.

[2]Fat mixtures for the diets were: D - 100% hydrogenated coconut oil; E - 50% hydrogenated coconut oil + 50% coconut oil; F - 67% coconut oil + 33% corn oil; G - 100% corn oil.

[3]The first number of the fatty acid designation represents the carbon chain length, and the number following the colon stands for the number of double bonds. The 'ω' number combination indicates the position of first double bond from the methyl end of the chain.

After the feeding period, the rats were anesthetized with diethyl ether and were decapitated. The blood was recovered by letting it drain for 30 seconds into a bottle containing heparin to prevent clotting. The heart

and liver tissues were dissected out, rinsed with cold water, wiped dry and weighed. After weighing, the tissues were placed immediately in individual bottles containing 0.9% saline solution with enough headspace to allow for expansion during freezing and stored in a -20°F freezer until extraction.

Extraction Method

The tissues in each treatment were subdivided into three groups. Each group of tissues was extracted using the methods of Folch et al. (1957). This consisted of homogenizing each gram of tissue with 17 parts of a 2:1 (v/v) chloroform:methanol solution for three minutes. The homogenate was filtered through a Whatman filter paper No. 1 and the filtrate transferred into a separatory funnel. Distilled water was added in the amount of 2/10 the volume of the extract and the mixture was stirred. The mixture was allowed to stand for about 5-6 hours until it separated into clear layers. The clear bottom layer containing the lipids was drained from the separatory funnel and evaporated to near dryness using a rotatory vacuum evaporator.

In the first experiment, the sample was dissolved with chloroform:methanol (2:1, v/v), dried over anhydrous sodium sulfate and transferred into vials fitted with screw caps. Chloroform was added to adjust the volume.

A piece of dry ice was also added to displace the air in the headspace before putting the caps on.

This procedure was varied for the second trial. Instead of taking up the extract in chloroform:methanol solution, only chloroform was used. Prior to storage, the solution was made up 2 parts chloroform to 1 part hexane by volume and butylated hydroxytoluene (BHT) was added as an antioxidant. All samples were stored at -20°F.

Thin-Layer Chromatography

Separation of phospholipids into classes was accomplished primarily by thin-layer chromatography. This technique allowed for a simple and rapid separation not available with other methods although the amount of sample was limited.

The plates used were 20 cm x 20 cm ordinary window glass which were cleaned thoroughly with soap and water and rinsed with acetone. Fifty grams of silica gel G were slurried with 100 ml of water and spread to a thickness of 0.5 cm over 5 plates. The plates were air dried and activated for one hour at 100°C before use.

For the one-dimensional TLC, the sample was streaked along one side of the plate and developed in a solvent system consisting of chloroform:methanol:28% ammonium hydroxide in a ratio of 65:25:4 by volume. This permitted the separation of pure phosphatidylethanolamine

(PE) and phosphatidylcholine (PC) as shown in Figure 1. The acidic phospholipids concentrated near the origin and did not interfere in the separation especially of phosphatidylserine which moved with PE in the neutral solvent system.

Because of the inability to separate all the phospholipid classes in one dimensional TLC, two dimensional TLC was used. This consisted of developing the plates in one direction and then, after drying, developing the plates 90° to the first direction in another solvent system.

In the first feeding trial samples, separation was accomplished using the following solvent systems: chloroform:methanol:water, 65:25:4 (v/v) for the first dimension chromatography and chloroform:methanol:water, 55:25:4 (v/v) for the second. After the samples were applied, the plates were exposed to ammonia vapor for about 10-15 seconds before development in the other direction.

The variability in the separation of the systems used in the first feeding trial prompted the shift to another solvent system. This new solvent system which was patterned after Rouser (1961) and modified by Parsons and Patton (1967) gave a more consistent result (Figure 2). In the first dimension, chloroform:methanol:water: ammonium hydroxide at a ratio of 130:70:8:0.5 (v/v/v/v) was used followed by chloroform:acetone:methanol:acetic

Figure 1.--Separation of total phospholipids of rat heart by one-dimensional thin-layer chromatography using a solvent system made up of chloroform:methanol:ammonium hydroxide (65:25:4, v/v/v). Abbreviations: NL--neutral lipids, CL--cardiolipin, PE--phosphatidylethanolamine, PC--phosphatidylcholine, SPH--sphingomyelin, PI--phosphatidylinositol, PS--phosphatidylserine, LPC--lysophosphatidylcholine.

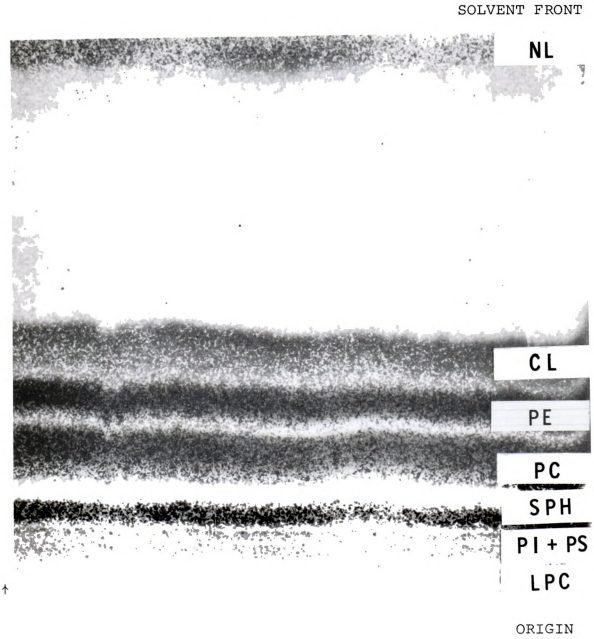


Figure 1

Figure 2.--Two-dimensional thin-layer chromatography of total lipid extract of rat liver using silica Gel HR (0.5 mm.). The plate was developed in direction A with chloroform:methanol:water: ammonium hydroxide (130:70:8:0.5 by volume) followed by air drying and development in the direction B with chloroform:acetone:methanol: acetic acid (60:20:20:20 by volume). Abbreviations: NL--neutral lipids, CL--cardiolipin, PE--phosphatidylethanolamine, PI--phosphatidylinositol, PC--phosphatidylcholine, SPH--sphingomyelin, PS--phosphatidylserine, LPC--lysophosphatidylcholine.

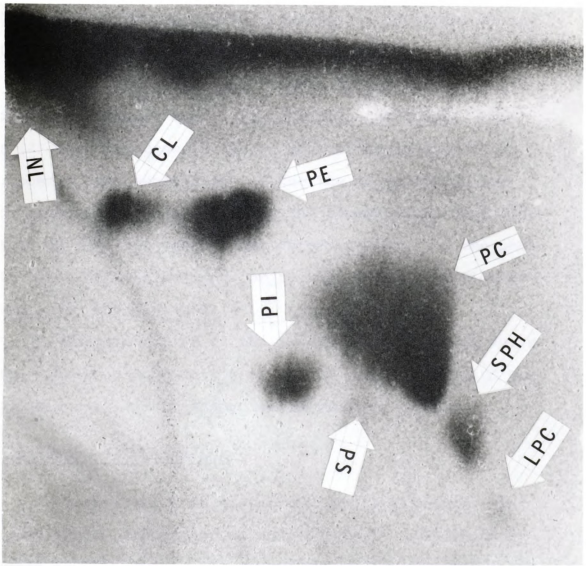


Figure 2

acid (60:20:20:20 v/v/v/v) in the other direction. Development time was about 30 minutes in the first and about 50 minutes in the second development. A 10-minute drying time was used between developments.

The spots were made visible by spraying with (1) molybdate-sulfuric acid spray (Dittmer and Lester, 1964) or (2) 0.2% fluorescein dye in ethanol depending on the purpose of the separation. Since the molybdate is destructive, it is used only when the phospholipid separated is to be used for phosphorous determination. If the phospholipids were to be used for further study, the flourescein spray was employed.

Identification of the phospholipid bands or spots were made by a combination of sprays, use of standard compounds and IR spectroscopy (see Appendices A and B).

Phosphorous Determination

Quantitative determination of phospholipids was accomplished by phosphorous analysis using the method of Rouser et al. (1966). It consisted of directly digesting the silica gel containing the phospholipid without elution. The phospholipids, after separation on TLC, were made visible using the molybdate spray. The spots were scraped off the plates into 30 ml Kjeldahl flasks. A 0.9 ml portion of 72% perchloric acid was added and the samples digested for 20-30 minutes. The digested samples

were allowed to cool and 5 ml portions of distilled water were added to each flask. This was followed by 1 ml each of 2.5% ammonium molybdate and 10% ascorbic acid solutions. The total volume was finally adjusted to 10 ml with the addition of 2 ml water which was also used to rinse the neck of the flasks. The color was developed by placing the flask in a boiling water bath for 5 minutes followed by cooling. The contents of the flasks were transferred to centrifuge tubes to spin down the silica gel before the absorbancy reading was made at 820 mμ.

The phosphorous content of the samples was determined by referring the measured absorbancy to a standard curve of absorbance vs concentration of phosphorous. The standard curve was prepared using a standard phosphorous solution plus silica gel and analyzed in a manner similar to that of the samples. The addition of silica gel G to the standard phosphorous solution was necessary since the slope of the curve with and without silica gel G was different.

Enzyme Hydrolysis

Phospholipase A was used to determine the fatty acid distribution in the phospholipid molecule. The enzyme is specific for the ester linkage in the β-position.

The phospholipase assay by Yabuchi and O'Brien (1968) was used. The phospholipid separated by TLC was

extracted with 2:1 chloroform:methanol from the silica gel. The combined extract was diluted with water equivalent to the amount of methanol in the extract to allow for the separation of the chloroform layer containing the lipids. The chloroform was evaporated to dryness and the residue taken up with 5 ml of diethyl ether. The enzyme dissolved in a 0.1M borate buffer containing calcium chloride was added at the rate of 0.4 ml per tube. The enzyme concentration was 4 mg/ml while that of calcium chloride was 1.0×10^{-3}M. The reaction was carried out at 27°C for 5-6 hours with constant shaking. For the hydrolysis of PE, the reaction mixture was made basic with the addition of a drop of 0.1N KOH solution.

The reaction was stopped with the addition of 1:1 chloroform:methanol which was also used for the extraction of the phospholipids. After concentrating the extracts, the components were separated on TLC and the bands corresponding to the fatty acids and the lysophospholipids were scraped off. The lysophospholipids were interesterified using the low temperature-KOH method (Zook, 1968) and the free fatty acids were methylated by diazomethane (Baer and Maurukas, 1955).

Preparation of Methyl Esters

Methyl esters from phospholipids were prepared according to the method of Zook (1967). The phospholipid

separated by thin-layer chromatography was scraped off the plates and placed into 150 ml Erlenmeyer flasks containing 20 ml diethyl ether. The solution was cooled to -60°C in a dry ice-acetone bath while under constant agitation. A 15 ml portion of absolute methanol was added followed by 4 grams KOH dissolved in 15 ml methanol. The reaction mixture was cooled to -50°C and then taken out of the bath and stirred while warming to room temperature.

The mixture was transferred to a 500 ml separatory funnel with 300 ml distilled water and extracted with 20, 15 and 10 portions of petroleum ether. The extracts were combined and washed with distilled water and dried over anhydrous sodium sulfate. The supernatant was concentrated under nitrogen for gas chromatographic analysis.

A fast and simple method using diazomethane was employed to prepare methyl esters of free fatty acids. Diazomethane was first prepared according to the method of Arndt (1943) in which N-nitrosomethylurea was reacted with a 50% KOH solution which was overlayered with diethyl ether. The evolving diazomethane was absorbed in the ether layer. The yellow ethereal solution was pipetted into a tube containing KOH pellets to remove residual moisture. This reagent was added to the free fatty acids until a residual yellowish tint appeared

indicating the completion of the reaction. The solvent was then evaporated to dryness under nitrogen and the ester residue taken up in petroleum ether for chomatographic analysis.

Gas Chromatography

Gas chromatography of the methyl esters and dimethylacetal was carried out on a Beckman Model GC-5 gas chromatograph equipped with flame ionization and thermal conductivity detectors. Separation of the esters was effected on two 1/8" x 6' columns packed with Chromosorb W coated with 20% diethylene glycol succinate (DEGS) with 1% phosphoric acid. All gas chromatographic analyses were carried out using a flame ionization detector coupled to a 10 inch recorder. The following operating conditions were used: column temperature, 185°C; helium flow, 60 cc/min; hydrogen flow, 20 cc/min; air flow, 250 cc/min; detector temperature, 250°C.

The fatty acid esters and dimethyl acetals were identified by comparison of the relative retention times with those of the standards as well as those published. Percentage composition was based on the peak areas obtained by multiplying the retention time with the peak height. The fatty acids with more than twenty carbon chain length and more unsaturated than arachidonic acid were not included in the calculation of the percentage

composition of the fatty acids because they were present in trace amounts.

Plasmalogen Analysis

Determination of plasmalogen content of the different phospholipids was accomplished by the use of phosphorous analysis. The phospholipid sample dissolved in a 0.1 ml of 1:1 chloroform:methanol was treated with 1 ml of a solution of 13 mg/ml 2,4-dinitrophenylhydrazine in 55% phosphoric acid. The plasmalogen was hydrolyzed by the acid resulting in the liberation of the aldehydes which combined with the 2,4-dinitrophenylhydrazine to form hydrazones. The mixture was reacted for 1.5 hours under constant agitation. After the reaction, the mixture was diluted with 10 ml distilled water and extracted with hexane. The extract containing the hydrazones, diacyl-and lysophospholipids was concentrated and spotted on TLC plates for separation. The phosphorous content of the diacyl- and the lysocompound was determined as a measure of the plasmalogen content. The hydrazones were separated for the regeneration of the aldehydes. The scheme for the determination is as follows:

$$\text{Plasmalogen} + H_2O \xrightarrow[H^+]{} \text{Aldehyde} + \text{lysophospholipid}$$

$$\text{Diacylphospholipid} + H_2O \xrightarrow[H^+]{} \text{No reaction}$$

$$\text{Aldehyde} + \text{2,4-DNPHydrazine} \longrightarrow \text{2,4-DNPHydrazone}$$

$$\% \text{ Plasmalogen} = \frac{\mu g \text{ P (lysophospholipid)}}{\mu g \text{ P (diacyl- + lysophospholipid)}} \times 100$$

Preparation of Dimethylacetal (DMA)

The 2,4-dinitrophenylhydrazine derivative of the aldehyde could be converted directly to the dimethylacetal using the method of Viswanathan et al. (1967). The hydrazones were dissolved in 25 ml diethyl ether and then cooled to 10°C. Two ml concentrated H_2SO_4 was added followed by 15 ml of levulinic acid. The mixture was reacted for one hour after which a 15 ml portion of absolute methanol was added followed with 15 ml of 35% KOH in methanol. The reaction was allowed to proceed for another 10 minutes before the mixture was extracted 3 times with hexane to recover the DMA. The extracts were then combined, dried over anhydrous sodium sulfate and concentrated under nitrogen gas for GLC analysis.

RESULTS AND DISCUSSION

Growth Rate

The average weekly weights of the rats during the two feeding trials are shown in Tables 4 and 5. The first feeding trial showed that the rats raised on a diet

Table 4.--Average weekly weights[1] of rats fed with diets containing different levels of essential fatty acids from the first feeding trial.

Diets	Linoleic acid as % of total dietary calories	Number of Weeks					
		0	1	2	3	4	5
		grams					
A	0.25	46.0	81.8	119.1	156.5	199.1	231.2[a]
B	6.00	46.1	79.4	106.4	142.2	181.4	205.6[b]
C	12.00	42.8	76.8	112.6	149.9	194.9	216.1[c]

[1]Means in the final week followed by the same superscript are not significantly different at the 5% level as determined by the Duncan Multiple Range Test.

containing coconut oil attained the highest average weight compared to the rats fed either corn oil or the mixture of the two oils. The differences in the final average weights were significant at the level of 5%. It would appear that coconut oil is a better fat supplement

Table 5.--Average weekly weights[1] of rats raised on a standard diet containing different levels of linoleic acid from the second feeding trial.

Diets	Linoleic acid as % of total dietary calories	Number of Weeks					
		1	2	3	4	5	6
		grams					
D	0.0	55.5	95.9	139.6	172.2	214.5	246.1	273.0[a]
E	0.5	55.8	90.7	134.5	180.5	201.0	245.0	275.0[a]
F	4.0	54.4	97.7	139.3	174.1	222.3	265.5	292.0[b]
G	12.0	55.5	96.1	142.9	182.5	232.0	296.0	307.0[c]

[1]Means in the final week followed by the same superscrips are not significantly different at the 1% level as determined by the Duncan Multiple Range Test.

for growth than corn oil or the mixture of corn and coconut oil. This is contrary to the fact that corn oil by virtue of its high linoleic acid content should be a better dietary fat than coconut oil. It has to be noted, however, that during the first feeding trial several animals appeared to be in poor health in the lots given corn oil (5 out of 20 animals) and the oil mixture (2 out of 20 animals) as indicated by loss of weight. Whether this reflects the general condition of the animals in these lots is difficult to say.

In contrast, the second feeding trial showed that the weight gains paralleled the amount of essential fatty acids (EFA) in the diet. The highest weight was attained by the animals given corn oil followed by those animals receiving diets in the order of decreasing amounts of EFA. Statistical analysis of the final weights attained showed no significant differences between those rats given hydrogenated coconut oil and those given the 1:1 mixture of hydrogenated coconut oil and plain coconut oil. These two lots have diets which contain EFA below the minimum requirement for male rats. Beyond this minimum level (1%), the weight differences became significant. Weight gains became proportionally high with the increase of linoleic acid in diets F and G. This result is in agreement with those reported in the literature (Hill et al., 1957; Walker, 1967) wherein higher weight gains were obtained in animals receiving higher amounts of essential fatty acids from the diet.

Comparison of the weights of the animals between two feeding trials was not possible except with the rats receiving the diet containing corn oil. At the fifth week of feeding, there was a great difference between the weights attained for both feeding trials. In the first experiment the rats had an average weight of 216.1 g while in the second experiment the average weight was 296.0 g.

It may be speculated that the differences in the physiological state of the animals as affected by change of environmental conditions or other unfavorable conditions may be greater in the first experiment than in the second trial so as to offset the influence of the diet.

Phospholipid Class

The phospholipid composition of liver and heart are shown in Tables 6 to 9. In the first feeding trial, the liver lipids (Table 6) showed a predominance of PC and PE among the phospholipids. Phosphatidycholine varied from 51.4% to 52.7% while PE ranged from 23.8% to 27.0%. The other phospholipids were all present at levels below 10% each. The different levels of linoleic acid in the basal diet appeared to have affected the PI and sphingomyelin fraction. Significant differences were obtained among the three diets used. The amount of PI appeared to be enhanced in diets containing high amounts of linoleic acid while sphingomyelin was reduced. The other phospholipids did not vary appreciably. Analyses for the second feeding trial (Table 7), however, did not confirm the results found in the first experiment as far as PI or sphingomyelin was concerned. No significant differences were obtained in the levels of the phospholipids from rats raised on a diet containing different levels of linoleic acid.

Table 6.--Phosphilipid composition[1] of total liver lipid extract from rats raised on diets containing different levels of linoleic acid.[2]

Phospholipid Class	Dietary Linoleate, % of Total Calories in the Diet		
	0.5	6.0	12.0
Cardiolipin	5.3[a][3]	5.7[a]	5.2[a]
Phosphatidyl-ethanolamine	23.8[a]	26.4[a]	27.0[a]
Phosphatidyl-choline	52.7[a]	51.4[a]	52.0[a]
Phosphatidyl-serine	2.7[a]	2.4[a]	1.4[a]
Phosphatidyl-inositol	3.8[a]	6.6[b]	9.0[c]
Sphingomyelin	8.3[a]	5.1[b]	3.0[c]
Lysophosphatidy-choline	2.6[a]	2.3[a]	2.5[a]

[1]Percentage composition based on phosphorous determination.

[2]First feeding trial.

[3]Means of a given phospholipid followed by the same superscript are not significantly different at the 5% level as determined by the Duncan Multiple Range Test.

Heart phospholipids did not exhibit any apparent response with the dietary fat in either feeding trial (Tables 8 and 9). The levels of PC and PE were slightly different from those of the liver. In the heart extract, PE and PC appeared in almost equivalent amount and still constituted the bulk of the phospholipids. The primary

Table 7.--Phospholipid composition[1] of the total lipid extract of liver from rats raised on a standard diet containing different levels of dietary linoleate.[2]

Phospholipid Class	Dietary Linoleate, % of Total Calories in the Diet			
	0.0	0.5	4.0	12.0
Cardiolipin	4.8[a3]	5.6[a]	5.5[a]	4.9[a]
Phosphatidyl-ethanolamine	24.2[a]	24.3[a]	27.4[a]	27.5[a]
Phosphatidyl-choline	53.7[a]	51.4[a]	52.1[a]	50.2[a]
Phosphatidyl-inositol	7.1[a]	9.4[a]	7.9[a]	8.2[a]
Phosphatidyl-serine	3.4[a]	2.9[a]	2.3[a]	1.7[a]
Sphingomyelin	4.2[a]	3.2[a]	3.4[a]	5.0[a]
Lysophosphatidyl-choline	2.6[a]	3.1[a]	2.0[a]	2.6[a]

[1]Percentage composition based on phosphorous determination.

[2]Second feeding trial.

[3]Means of a given phospholipid followed by the same superscript are not significantly different at the level of 5% as determined by the Duncan Multiple Range Test.

difference lies on the level of cardiolipin where higher amounts were obtained in the second trial than in the first feeding trial.

The phospholipid class composition found for the liver agreed with those reported in literature (Getz et

Table 8.--Phospholipid composition[1] of total lipid extract of rat heart as affected by different levels of linoleic acid in the diet.[2]

Phospholipid Class	Dietary Linoleate, % of Total Calories in the Diet		
	0.5	6.0	12.0
Cardiolipin	9.6[a3]	11.6[a]	12.1[a]
Phosphatidylethanolamine	38.8[a]	35.2[a]	34.7[a]
Phosphatidylcholine	38.7[a]	38.6[a]	42.3[a]
Phosphatidylserine	2.5[a]	2.4[a]	1.6[a]
Phosphatidylinositol	4.1[a]	3.4[a]	3.2[a]
Sphingomyelin	3.5[a]	4.4[a]	3.9[a]
Lysophosphatidylcholine	2.8[a]	4.3[b]	2.2[b]

[1]Percentage composition based on phosphorous analysis.

[2]First feeding trial.

[3]Means of a given phospholipid followed by the same superscript are not significantly different at the level of 5% as determined by the Duncan Multiple Range Test.

al., 1962; Cuzner and Davison, 1967). Cuzner and Davison (1967) reported the predominance of PC (57.0%) and PE (24.7%) in liver homogenates. The minor phospholipid content found in the present study are within the range of values reported.

Heart phospholipids contained less PC than the liver. There was also a higher amount of cardiolipin in

ble 9.--Phospholipid composition[1] of total heart lipid extract from rats fed with a basal diet containing different levels of linoleic acid.[2]

Phospholipid Class	Dietary Linoleate, % of Total Calories in the Diet			
	0.0	0.5	4.0	12.0
rdiolipin	17.8[a3]	15.1[a]	16.5[a]	16.9[a]
osphatidyl-ethanolamine	32.1[a]	31.6[a]	33.4[a]	32.8[a]
osphatidyl-choline	35.0[a]	38.2[a]	36.8[a]	36.2[a]
osphatidyl-inositol	4.3[a]	5.1[a]	4.7[a]	3.9[a]
osphatidyl-serine	3.8[a]	3.3[a]	2.6[a]	3.6[a]
hingomyelin	3.7[a]	4.8[a]	3.9[a]	3.5[a]
sophosphatidyl-choline	3.2[a]	1.9[a]	2.5[a]	3.1[a]

[1]Percentage composition based on phosphorous alysis.

[2]Second feeding trial.

[3]Means of a given phospholipid followed by the me superscript are not significantly different at the level as determined by the Duncan Multiple Range Test.

e heart compared to the liver. The values found in the esent work on the relative proportion of heart cardiopin, PE and PC agreed with those reported in literare (Getz et al. 1962).

The influence of different dietary fat on the ospholipid composition has been studied in many

stances (Neudoerffer and Lea, 1967; Sinclair, 1929). udoerffer and Lea (1967) reported that dietary fat affected only the neutral lipids in turkey muscle and did ot influence the proportion and concentration of the ndividual phospholipids. Differences in proportion appeared to be more prominent between tissues. This had een observed by others (Jacobs et al., 1950; Sheltawy nd Dawson, 1966) which brought about speculation on a ossible role for phospholipids in tissues. Neudoerffer nd Lea (1967) noted that there was a high proportion of E, PS and PI and low PC in physically more active leg uscle than in breast muscle. These differences also ccurred between the liver and the heart. In addition here was a higher level of cardiolipin in heart than in iver. The level of cardiolipin, which is a major phospholipid of the mitochondria, probably reflects its role ndirectly in active muscle which contains a high density f mitochondria (Sheltawy and Dawson, 1966).

Fatty Acid Composition

ajor Phospholipids

hosphatidylcholine (PC)

The fatty acid composition of rat liver PC from he second feeding trial is shown in Table 10. A large mount of unsaturated fatty acids belonging to the oleic

able 10.--Fatty acid composition[1] of liver phosphatidylcholine from rats fed different levels of linoleic acid in the diet.[2]

Fatty Acids[3]	Dietary Linoleate, % of Total Calories in the Diet			
	0.0	0.5	4.0	12.0
6:0	25.6[a][4]	22.6[a]	25.9[a]	26.1[a]
6:1 ω 7	5.9[a]	4.4[a]	3.0[a]	1.4[a]
8:0	24.5[a]	27.1[a]	24.4[a]	22.0[a]
8:1 ω 9	18.3[a]	15.4[a]	10.7[b]	6.8[c]
8:2 ω 6	4.2[a]	7.0[a]	12.7[b]	16.1[c]
0:3 ω 9	14.0[a]	11.5[a]	1.0[b]	0.3[b]
0:4 ω 6	7.5[a]	11.7[a]	22.5[b]	27.3[c]
aturates	50.1	49.7	48.3	48.1
nsaturates	49.9	50.0	51.7	51.9

[1]Peak area percent.

[2]Second feeding trial.

[3]The first number of the fatty acid designation epresents the carbon chain length and the number following the colon stands for the number of double bonds. The ω' number combination indicates the position of the irst double bond from the methyl end of the chain.

[4]Means of a given fatty acid followed by the same uperscript are not significantly different at the 5% evel as determined by the Duncan Multiple Range Test.

acid family were present in rats fed the EFA-free diet (Diet D). These were eicosatrienoic acid (20:3 w 9) and oleic acid (18:1 w 9) which amounted to 14.0% and 18.3% respectively. Linoleic acid (18:2 w 6) and arachidonic acid (20:4 w 6) were low. Palmitoleic acid (18:1 w 7) appeared at a level of 5.9%. At the highest level of linoleic acid in the diet (Diet G), the members of the oleic acid family and palmitoleic acid were low while those of the linoleic acid group were high. The saturated stearic and palmitic acids remained practically constant in the liver PC from the rats raised on the different dietary regimens. These differences in the fatty acid composition of liver PC brought about by the differences in the amount of dietary linoleate confirmed the results found in the first experiment (Table 11).

The total amount of unsaturated fatty acids in heart PC (55.4 to 58.2%) was slightly more than the total of the saturated fatty acids. The proportions of the unsaturated fatty acids of heart PC from rats raised on diet G (Table 12) approached those of the liver PC. Linoleic acid and arachidonic acid occurred at a level of 17.4% and 30.2% respectively, while oleic acid was about 8.0%. Eicosatrienoic acid, a metabolite of oleic acid, was absent. Stearic acid (27.4%) level was more than the palmitic acid (16.7%). The absence of EFA in diet D resulted in low amounts of arachidonic acid (6.0%)

Table 11.--Fatty acid composition[1] of liver phosphatidylcholine from rats given different levels of linoleic acid in the diet.[2]

Fatty Acids[3]	Dietary Linoleate, % of Total Calories in the Diet		
	0.5	6.0	12.0
16:0	25.5[a4]	24.6[a]	17.4[a]
18:0	22.4[a]	25.8[a]	17.0[a]
18:1 ω 9	24.4[a]	22.4[a]	24.5[b]
18:2 ω 6	9.1[a]	11.3[b]	20.7[c]
20:3 ω 9	10.8[a]	8.9[a]	2.0[b]
20:4 ω 6	7.7[a]	6.9[a]	18.1[b]

[1]Peak area per cent.

[2]First feeding trial.

[3]The first number of the fatty acid designation represents the carbon chain length and the number following the colon stands for the number of double bonds. The 'ω' number combination indicates the position of the first double bond from the methyl end of the chain.

[4]Means of a given fatty acid followed by the same superscript are not significantly different at the 5% level as determined by the Duncan Multiple Range Test.

and linoleic acid (5.8%) and high amounts of oleic acid (27.9%) and eicosatrienoic acid (12.9%) in the tissue. The heart PC from rats in the first experiment (Table 13) exhibited similar variations in the fatty acid composition.

Based on the fatty acid composition of the PC fraction from both tissues of the rats fed with a corn

ble 12.--Fatty acid composition[1] of heart phosphatidylcholine from rats fed with a basal diet containing different amounts of linoleic acid.[2]

Fatty Acids[3]	Dietary Linoleate, % of Total Calories in the Diet			
	0.0	0.5	4.0	12.0
6:0	20.2[a][4]	19.4[a]	20.1[a]	16.7[a]
6:1 ω 7	3.5[a]	2.9[a]	trace[5]	trace
8:0	23.7[a]	23.5[a]	24.7[a]	27.4[a]
8:1 ω 9	27.9[a]	26.2[b]	11.3[c]	8.0[c]
8:2 ω 6	5.8[a]	9.3[b]	17.3[c]	17.4[c]
0:3 ω 9	12.9[a]	10.4[b]	0.9[c]	trace
0:4 ω 6	6.0[a]	10.4[b]	25.9[c]	30.2[d]
aturates	43.9	41.9	44.6	44.1
nsaturates	56.1	58.2	55.4	55.6

[1]Peak area percent.

[2]Second feeding trial.

[3]The first number of the fatty acid designation epresents the carbon chain length and the number following the colon stands for the number of double bonds. The ω' number combination indicates the position of the irst double bond from the methyl end of the chain.

[4]Means of a given fatty acid followed by the same uperscript are not significantly different at the 5% evel as determined by the Duncan Multiple Range Test.

[5]Less than 0.1% of total peak area.

ıble 13.--Fatty acid composition[1] of heart phosphatidylcholine from rats fed a standard diet containing different amounts of linoleic acid.[2]

ıtty Acid[3]	Dietary Linoleate, % of Total Calories in the Diet		
	0.5	6.0	12.0
6:0	18.4[a4]	19.0[a]	20.9[a]
8:0	23.7[a]	21.5[a]	19.4[a]
8:1 ω 9	24.1[a]	16.0[b]	13.1[c]
8:2 ω 6	12.2[a]	16.0[b]	17.3[b]
0:3 ω 9	11.5[a]	5.1[b]	0.3[c]
0:4 ω 6	10.0[a]	22.4[b]	28.9[c]

[1]Peak area per cent.

[2]First feeding trial.

[3]The first number of the fatty acid designation epresents the carbon chain length and the number following the colon stands for the number of double bonds. The ω' number combination indicates the position of the first ouble bond from the methyl end of the chain.

[4]Means of a given fatty acid followed by the same uperscript are not significantly different at the 5% evel as determined by the Duncan Multiple Range Test.

il supplemented diet would indicate that phospholipid pecies of heart tissue contained more of the stearic cid containing species which contained palmitic acid. he reverse was true in the case of liver PC which indiates the selectivity of the enzymes for the synthesis of he phospholipids. Heart PC contained slightly lower

amounts of saturated fatty acids than unsaturated fatty acids. Since there are only two positions available for the two fatty acids and most of the unsaturated fatty acids are in the β-position of the phospholipid molecule (Hanahan, 1967), it would indicate that some of the unsaturated fatty acids would be in the α-position. The unsaturated fatty acids present in the α-position were mostly oleic and palmitoleic acids as was shown by other workers (Pudelkewics and Holman, 1968; Van Golde et al., 1968).

Phosphatidylethanolamine (PE)

PE varied similarly to PC in fatty acid composition in relation to the dietary essential fatty acid. The liver PE of rats fed the corn oil-containing diet in the second feeding trial (Table 14) contained an almost even 50% saturated fatty acids and 50% unsaturated fatty acids. Of the unsaturated fatty acids, 34.4% consisted of arachidonic acid, while oleic and linoleic acids were present at 5.8% and 9.4% respectively. Eicosatrienoic acid was absent. Palmitic acid and stearic acid were the only saturated fatty acids present at the levels of 19.8% and 30.3% respectively. In the case of heart PE from rats given hydrogenated coconut oil, the amounts of linoleic acid (3.6%) and arachidonic acid (20.7%) were much lower than those found in the animals

Table 14.--Fatty acid composition[1] of liver phosphatidylethanolamine from rats fed a basal diet containing different levels of linoleic acid.[2]

Fatty Acids[3]	Dietary Linoleate, % of Total Calories in the Diet			
	0.0	0.5	4.0	12.0
16:0	22.3[a][4]	24.4[a]	23.0[a]	19.8[a]
16:1 ω 7	2.8[a]	2.8[a]	0.8[b]	0.2[b]
18:0	28.4[a]	29.0[a]	30.0[a]	30.3[a]
18:1 ω 9	11.3[a]	9.9[b]	5.9[c]	5.8[c]
18:2 ω 6	2.0[a]	3.0[b]	7.4[c]	9.4[d]
20:3 ω 9	14.0[a]	7.9[b]	0.3[c]	trace[5]
20:4 ω 6	19.2[a]	22.9[b]	32.8[c]	34.4[c]
Saturates	50.7	53.4	53.0	50.1
Unsaturates	49.3	46.5	47.2	49.8

[1]Peak area percent.

[2]Second feeding trial.

[3]The first number of the fatty acid designation represents the carbon chain length, and the number following the colon stands for the number of double bonds. The 'ω' number combination indicates the position of the first double bond from the methyl end of the chain.

[4]Means of a given fatty acid followed by the same superscript are not significantly different at the 5% level as determined by the Duncan Multiple Range Test.

[5]Less than 0.1% of total peak area.

fed the corn oil-containing diet. On the other hand, eicosatrienoic acid (20.8%) and oleic acid (13.6%) occurred in higher amounts. The variation in the fatty acid composition of liver PE as influenced by the dietary linoleate was also exhibited at the intermediate levels. This response confirmed the results found in the first feeding trial (Table 15). The main difference lies in the levels of the different fatty acids, especially eicosatrienoic acid where lower amounts were found in the first experiment than in the second. The discrepancy may be explained by the fact that ordinary coconut oil which contained a small amount of linoleic acid was used in the first experiment while hydrogenated coconut oil was used in the second trial. In addition the second experiment was carried out a week longer.

Heart PE (Table 16) from the second feeding experiment had a similar fatty acid variation with respect to the diet but differed in the levels at which each fatty acid occurred. Heart PE from rats given diet G was characterized by a higher stearic acid (36.9%) content than palmitic acid (10.2%). The unsaturated fatty acids, however, were present in amounts close to that of the liver PE. Heart PE contained 29.9% arachidonic acid and 15.1% linoleic acid. Eicosatrienoic and palmitoleic acids were absent. The low level of linoleic acid in diet D resulted in reduced levels of arachidonic and

Table 15.--Fatty acid composition[1] of liver phosphatidylethanolamine from rats given different levels of linoleic acid in the diet.[2]

Fatty Acid[3]	Dietary Linoleate, % of Total Calories in the Diet		
	0.5	6.0	12.0
16:0	29.2[a4]	21.7[b]	18.2[b]
18:0	28.0[a]	25.8[a]	20.8[a]
18:1 ω 9	12.5[a]	10.0[b]	7.1[c]
18:2 ω 6	4.0[a]	15.0[b]	13.3[b]
20:3 ω 9	4.8[a]	2.0[b]	trace[5]
20:4 ω 6	21.6[a]	25.8[b]	40.5[c]

[1]Peak area per cent.

[2]First feeding trial.

[3]The first number of the fatty acid designation represents the carbon chain length and the number following the colon stands for the number of the double bonds. The 'ω' number combination indicates the position of the first double bond from the methyl end of the chain.

[4]Means of a given fatty acid followed by the same superscript are not significantly different at the 5% level as determined by the Duncan Multiple Range Test.

[5]Less than 1% of total peak area.

linoleic acids while eicosatrienoic acid was elevated. The differences brought about by the dietary fat on the levels of the oleic and linoleic acid groups observed in the heart PE of rats from the second feeding trial also were observed in the first experiment (Table 17).

Table 16.--Fatty acid composition[1] of heart phosphatidylethanolamine from rats fed different amounts of linoleic acid in the diet.[2]

Fatty Acids[3]	Dietary Linoleate, % of Total Calories in the Diet			
	0.0	0.5	4.0	12.0
16:0	7.1[a][4]	7.6[a]	11.2[b]	10.2[b]
16:1 ω 7	1.9[a]	0.8[b]	trace[5]	trace
18:0	32.2[a]	31.2[a]	35.2[a]	36.9[a]
18:1 ω 9	13.6[a]	13.6[a]	10.6[a]	7.7[a]
18:2 ω 6	3.6[a]	5.1[b]	11.3[b]	15.1[b]
20:3 ω 9	20.8[a]	14.0[b]	0.2[c]	trace
20:4 ω 6	20.7[a]	27.4[b]	31.6[c]	29.9[c]
Saturates	39.9	38.8	46.4	47.1
Unsaturates	60.6	60.9	53.7	52.7

[1]Peak area percent.

[2]Second feeding trial.

[3]The first number of the fatty acid designation represents the carbon chain length, and the number following the colon stands for the number of double bonds. The 'ω' number combination indicates the position of the first double bond from the methyl end of the chain.

[4]Means of a given fatty acid followed by the same superscript are not significantly different at the 5% level as determined by the Duncan Multiple Range Test.

[5]Less than 0.1% of total peak area.

Table 17.--Component fatty acids[1] found in heart phosphatidylethanolamine from rats raised on diets containing different levels of linoleic acid.[2]

Fatty Acid[3]	Dietary Linoleate, % of Total Calories in the Diet		
	0.5	6.0	12.0
16:0	18.1[a4]	26.5[b]	25.0[b]
18:0	25.1[a]	22.3[a]	18.9[a]
18:1 ω 9	22.8[a]	6.9[b]	5.0[b]
18:2 ω 6	7.0[a]	14.1[b]	10.0[b]
20:3 ω 9	10.3[a]	2.9[b]	1.8[b]
20:4 ω 6	16.6[a]	27.3[b]	39.5[c]

[1]Peak area per cent.

[2]First feeding trial.

[3]The first number of the fatty acid designation represents the carbon chain length and the number following the colon stands for the number of double bonds. The 'ω' number combination indicates the position of the first double bond from the methyl end of the chain.

[4]Means of a given fatty acid followed by the same superscript are not significantly different at the 5% level as determined by the Duncan Multiple Range Test.

The amount of unsaturated fatty acids in both PE and PC belonging to the linoleic acid family increased proportionally with the amount of linoleic acid in the diet. In contrast, the use of pure EFA in the diet (Mohrhauer and Holman, 1963; Brenner and Jose, 1960)

brought about a proportional increase of the EFA and its metabolite in the tissue phospholipid over a certain range (0-1% of calorie) above which the amount of EFA in the phospholipid leveled off. This would indicate that the presence of non-essential fatty acids competes with the essential fatty acid for incorporation into the phospholipid. This also results in higher requirement for EFA in the presence of non-essential fatty acid.

From the works of Holman and Mohrhauer (1963) and Brenner and Peluffo (1966) it was indicated that linoleic acid inhibits the formation of eicosatrienoic acid by competing with oleic acid for the enzyme system responsible for the elongation reaction. This was inferred from the fact that in the presence of linoleic acid in the diet, eicosatrienoic acid either decreased or disappeared completely depending on the level of linoleic acid. The same groups (Brenner and Nervi, 1965; Mohrhauer and Holman, 1963) later showed that pure arachidonic acid also inhibited the formation of eicosatrienoic acid thus eliminating the possibility of inhibition by competition for enzyme of the elongation system. Both groups proposed that competition probably occurred at the level of acylation of the phospholipid. Johnson et al. (1967) provided some evidence in favor of the proposed mechanism by studying the kinetics of recovery from EFA deficiency. They found that there was 14-19 hours delay

in the appearance of arachidonic acid after feeding with linoleic acid. Simultaneously there was a decline of eicosatrienoic acid with the increase of linoleic acid. This competition could occur through the acyl transferase system found by Lands and Merkle (1963) where the fatty acid in the intact phospholipid is exchanged for another fatty acid or from de novo synthesis of phospholipid at the expense of the species already present.

Minor Phospholipids

Phosphatidylserine (PS)

Heart PS (Table 18) from rats given corn oil exhibited a high proportion of unsaturated fatty acid amounting to about 73.7%. Arachidonic acid amounted to 12.0% while linoleic acid accumulated to a level of 46.5%. At lower levels of linoleic acid in the diet, the amount of both linoleic acid and arachidonic acid became correspondingly low in the tissue. On the other hand, oleic acid and eicosatrienoic acid, which were low in rats fed with a corn oil-containing diet were present in high levels in tissues of rats on diet D. A peculiar variation occurred in the stearic fraction which was not found among the major phospholipids. Stearic acid occurred in heart PS of rats fed diet G (corn oil) at a level of 14.7% while in rats receiving a EFA deficient diet (D), it was present at 37.6%. This was accompenied by a

Table 18.--Fatty acid composition[1] of heart phosphatidylserine from rats given different levels of linoleic acid in the diet.[2]

Fatty Acids[3]	Dietary Linoleate, % of Total Calories in the Diet			
	0.0	0.5	4.0	12.0
16:0	14.4[a4]	7.2[b]	6.5[b]	11.5[c]
16:1 ω 7	4.8[a]	3.9[a]	trace[5]	1.5[b]
18:0	37.6[a]	25.9[b]	28.7[b]	14.7[c]
18:1 ω 9	21.5[a]	16.9[b]	10.5[c]	12.0[c]
18:2 ω 6	7.4[a]	16.8[b]	39.6[c]	46.5[d]
20:3 ω 9	7.7[a]	8.8[a]	1.1[b]	1.2[b]
20:4 ω 6	6.5[a]	10.4[a]	13.5[b]	12.7[b]
Saturates	52.0	33.1	35.2	26.2
Unsaturates	47.9	56.8	64.7	73.7

[1]Peak area percent.

[2]Second feeding trial.

[3]The first number of the fatty acid designation represents the carbon chain length, and the number following the colon stands for the number of double bonds. The 'ω' number combination indicates the position of the first double bond from the methyl end of the chain.

[4]Means of a given fatty acid followed by the same superscript are not significantly different at the 5% level as determined by the Duncan Multiple Range Test.

[5]Less than 0.1% of total peak area.

similar lowering of the amounts of palmitic acid in rats fed with higher amounts of linoleic acid as in diets E and F.

In the case of liver PS (Table 19) differences, particularly those of the unsaturated fatty acids, were observed between diets. Rats given diet G (corn oil) had less linoleic acid (17.8%) than arachidonic acid (28.6%) which is the reverse in heart PS. In lots receiving less linoleic acid, the amounts of both linoleic acid and arachidonic acid appeared in lower proportions and especially so in those receiving hydrogenated coconut oil. Eicosatrienoic acid, which was not present in the rats fed diets containing only corn oil, was about 14.3% in the rats given the diet containing hydrogenated coconut oil. Significant differences in the level of linoleic acid, arachidonic acid, eicosatrienoic acid and oleic acid occurred between diet E and F and F and G but not between D and E.

The saturated fatty acids of liver PS did not exhibit definite variation similar to that shown by heart PS. Stearic acid exhibited no significant differences in PS of rats given diet D, E, and F but diet G showed a significantly low level. On the other hand, the palmitic acid level of rats on diets D and E were similar but higher in rats on diet F and G.

Table 19.--Component fatty acids[1] of liver phosphatidylserine from rats raised on a basal diet containing different levels of linoleic acid.[2]

Fatty Acids[3]	Dietary Linoleate, % of Total Calories in the Diet			
	0.0	0.5	4.0	12.0
16:0	18.7[a4]	18.8[a]	26.4[b]	21.8[c]
16:1 ω 7	2.4[a]	1.6[a]	trace[5]	trace
18:0	36.0[a]	35.0[a]	34.0[a]	24.6[b]
18:1 ω 9	15.3[a]	15.7[a]	10.1[b]	7.1[c]
18:2 ω 6	3.9[a]	4.8[a]	12.0[b]	17.8[c]
20:3 ω 9	14.3[a]	12.6[a]	1.4[b]	trace
20:4 ω 6	9.4[a]	11.1[a]	16.4[b]	28.6[c]
Saturates	54.7	53.8	60.4	45.8
Unsaturates	45.3	45.8	39.9	54.1

[1]Peak area percent.

[2]Second feeding trial.

[3]The first number of the fatty acid designation represents the carbon chain length, and the number following the colon stands for the number of double bonds. The 'ω' number combination indicates the position of the first double bond from the methyl end of the chain.

[4]Means of a given fatty acid followed by the same superscript are not significantly different at the 5% level as determined by the Duncan Multiple Range Test.

[5]Less than 0.1% of total peak area.

Phosphatidylinositol (PI)

The dietary fat seems to be able to influence both saturated and unsaturated fatty acid in the case of the minor phospholipid of the phosphatidic acid ester type. As in liver PS, liver PI (Table 20) showed a significantly higher level of stearic acid (44.7%) from rats fed with diet D than PI from rats receiving diet G (35.0%). The over-all sum of the saturated fatty acid, however, remained constant among the four diets used because of the compensatory increase of palmitic acid. This would indicate a partial substitution of stearic acid containing PI with the palmitic acid containing species. Arachidonic acid and linoleic acid which amounted to 12.8% and 4.4% respectively in rats given diets with hydrogenated coconut oil, increased to 14.6% and 30.0% respectively in rats fed with corn oil supplemented diet. Eicosatrienoic acid and oleic acid varied from 18.8% and 8.2% in diet D to 1.4% and 6.1% in diet G respectively.

Heart PI (Table 21) exhibited similar variations in fatty acid composition as in the PI from liver especially in terms of oleic acid and linoleic acid content. Arachidonic acid varied significantly between diet D (9.6%) and diet G (22.3%), likewise linoleic acid was significantly different at diet D (1.2%) and diet G (13.3%). Differences at intermediate levels were

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Heart PI (Table 21) exhibited similar variations in fatty acid composition as in the PI from liver especially in terms of oleic acid and linoleic acid content. Arachidonic acid varied significantly between diet D (9.6%) and diet G (22.3%), likewise linoleic acid was significantly different at diet D (1.2%) and diet G (13.3%). Differences at intermediate levels were

Table 20.--Fatty acid composition[1] of liver phosphatidylinositol from rats fed a standard diet containing different amounts of linoleic acid.[2]

Fatty Acids[3]	Dietary Linoleate, % of Total Calories in the Diet			
	0.0	0.5	4.0	12.0
16:0	11.2[a4]	13.8[a]	13.9[a]	13.5[a]
16:1 ω 7	trace[5]	trace	0.7	trace
18:0	44.7[a]	39.6[a]	36.8[a]	35.0[a]
18:1 ω 9	8.2[a]	8.2[a]	7.1[a]	6.1[a]
18:2 ω 6	4.4[a]	6.8[b]	9.3[c]	14.6[d]
20:3 ω 9	18.8[a]	9.9[b]	5.7[b]	1.4[b]
20:4 ω 6	12.8[a]	21.2[b]	26.6[b]	30.0[b]
Saturates	55.9	52.4	51.7	50.2
Unsaturates	44.2	47.5	48.4	49.8

[1]Peak area percent.

[2]Second feeding trial.

[3]The first number of the fatty acid designation represents the carbon chain length, and the number following the colon stands for the number of double bonds. The 'ω' number combination indicates the position of the first double bond from the methyl end of the chain.

[4]Means of a given fatty acid followed by the same superscript are not significantly different at the 5% level as determined by the Duncan Multiple Range Test.

[5]Less than 0.1% of total peak area.

Table 21.--Component fatty acids[1] of rat heart phosphatidylinositol as affected by dietary fats.[2]

Fatty Acids[3]	Dietary Linoleate, % of Total Calories in the Diet			
	0.0	0.5	4.0	12.0
16:0	7.2[a4]	7.1[a]	7.0[a]	10.0[a]
16:1 ω 7	2.1[a]	1.3[a]	0.9[a]	1.4[a]
18:0	41.9[a]	42.4[a]	37.7[a]	40.7[a]
18:1 ω 9	18.4[a]	17.0[a]	13.0[b]	11.9[b]
18:2 ω 6	1.2[a]	2.2[a]	10.3[b]	13.3[c]
20:3 ω 9	22.4[a]	16.9[a]	2.1[b]	trace[5]
20:4 ω 6	9.6[a]	12.9[a]	29.2[b]	22.3[c]
Saturates	53.1	49.5	44.7	50.7
Unsaturates	46.9	50.3	55.5	48.9

[1]Peak area percent.

[2]Second feeding trial.

[3]The first number of the fatty acid designation represents the carbon chain length, and the number following the colon stands for the number of double bonds. The 'ω' number combination indicates the position of the first double bond from the methyl end of the chain.

[4]Means of a given fatty acid followed by the same superscript are not significantly different at the 5% level as determined by the Duncan Multiple Range Test.

[5]Less than 0.1% of total peak area.

significant between diet E and F and F and G but not between D and E. Oleic acid appeared to be at an almost constant level among the four diets but eicosatrienoic acid was low in rats receiving the high linoleic acid diets (F and G) and high in rats fed on diets D and E. The saturated fatty acids exhibited no significant differences among the four diets used.

Of the different classes of phospholipids, the minor components PI and PS have been the least studied. Most earlier studies were made on the composition of total phospholipids, cholesterol esters and neutral lipids and some on PC and PE. A number of reports on the fatty acid composition of both PI and PS from different animal tissues have been made. Peng and Dugan (1965) reported the fatty acid composition of PS from dark and light meat from normal chicken. They found large amounts of monoenoic acids particularly oleic acid at 19.17% and docosaenoic acid at 19.85% in dark meat. White meat had 42.54% oleic acid and roughly 10% each of stearic acid and arachidonic acid. Hanahan et al. (1964) found a highly unsaturated PS in human grey matter containing 36.6% docosahexaenoic acid and 21.5% oleic acid. The white matter PS contained essentially oleic acid and stearic acid at 47.6% and 43.5% respectively. Human red cell PS was found to have a high stearic acid (39.8%) and arachidonic acid (23.5%) content. Apparently comparison

of the fatty acid composition of a given phospholipid from one animal tissue to another is not possible because of the great biological variation. This indicates the specificity of the tissues in terms of each fatty acid composition. This is also true of PI. Human red cell PI has been reported to contain much higher palmitic acid (45.7%) than stearic acid (7.3%). It has no arachidonic acid but high amounts of tetraeicosaenoic acid (15.4%). It is a highly saturated phospholipid. In human plasma PI, however, stearic acid (31.5%) is at a higher level than palmitic acid (5.3%) and large amounts of arachidonic acid (24.4%) are present along with small amounts of other long chain fatty acids.

Johnson and co-workers (1967) analyzed the combined PS and PI fraction from EFA deficient rats 24 hours after being fed with safflower oil and reported no changes in the fatty acids. This is contrary to the findings in this experiment wherein both fractions were influenced by the dietary fat. This may be explained by the differences in the feeding experiments. While they killed animals 24 hours after feeding, the animals in the present experiment were kept for 6 weeks on the experimental diets. The differences in relative turnover of the phospholipid in the tissue have been recognized earlier (Collins, 1960; Chagraff, 1940; Perlman et al., 1939)

and even within a given class of phospholipid they are metabolized at different rates.

Cardiolipin

Cardiolipin presented an entirely different fatty acid composition from that of other phospholipids (Tables 22 and 23). Heart cardiolipin from rats given the diet containing corn oil showed 93.3% of the total fatty acids present as linoleic acid. The other fatty acids present were stearic and oleic acids at the levels of 2.0% and 4.8% respectively. It has no arachidonic acid nor eicosatrienoic acid. The heart cardiolipin from rats given EFA deficient diet (D) exhibited a low level of linoleic acid (59.8%) and high oleic acid content (25.1%). Stearic and palmitic acids were present in small quantities in rats fed coconut oil and even lower in corn oil fed rats.

Liver cardiolipin showed similar compositional variations with dietary fat as heart cardiolipin. Linoleic acid was present in liver cardiolipin in a slightly lower amount (79.3%) than the heart cardiolipin in the same diet. When the amount of dietary linoleate was reduced to zero, as in diet D, linoleic acid was present in cardiolipin at only 20.7%. Oleic acid which was present in the rats fed the corn oil-containing diet at 11.8%, appeared at a significantly higher level (49.0%) in the hydrogenated coconut oil fed rats. Stearic acid

Table 22.--Fatty acid composition[1] of heart cardiolipin from rats fed different levels of linoleic acid in the diet.[2]

Fatty Acids[3]	Dietary Linoleate, % of Total Calories in the Diet			
	0.0	0.5	4.0	12.0
16:0	5.2[a4]	6.7[a]	2.0[b]	trace[5]
16:1 ω 7	7.9[a]	8.2[a]	3.5[a]	trace
18:0	2.0[a]	3.0[a]	2.7[a]	2.0[a]
18:1 ω 9	25.1[a]	18.9[a]	12.9[a]	4.8[b]
18:2 ω 6	59.8[a]	63.2[a]	78.9[b]	93.3[c]
Saturates	7.2	9.7	4.7	2.0
Unsaturates	92.8	90.3	95.3	98.1

[1]Peak area percent.

[2]Second feeding trial.

[3]The first number of the fatty acid designation represents the carbon chain length, and the number following the colon stands for the number of double bonds. The 'ω' number combination indicates the position of the first double bond from the methyl end of the chain.

[4]Means of a given fatty acid followed by the same superscript are not significantly different at the 5% level as determined by the Duncan Multiple Range Test.

[5]Less than 0.1% of total peak area.

Table 23.--Fatty acid composition[1] of liver cardiolipin from rats fed different levels of linoleic acid in the diet.[2]

Fatty Acids[3]	Dietary Linoleate, % of Total Calories in the Diet			
	0.0	0.5	4.0	12.0
16:0	8.8[a4]	8.9[a]	7.3[a]	4.1[a]
16:1 ω 7	20.4[a]	14.9[b]	7.6[c]	3.1[c]
18:0	1.4[a]	2.3[a]	2.7[a]	1.4[a]
18:1 ω 9	49.0[a]	36.6[b]	15.8[c]	11.8[c]
18:2 ω 6	20.7[a]	37.2[b]	66.4[c]	79.3[c]
Saturates	10.2	11.2	10.0	5.5
Unsaturates	90.8	88.7	89.8	94.2

[1]Peak area percent.

[2]Second feeding trial.

[3]The first number of the fatty acid designation represents the carbon chain length, and the number following the colon stands for the number of double bonds. The 'ω' number combination indicates the position of the first double bond from the methyl end of the chain.

[4]Means of a given fatty acid followed by the same superscript are not significantly different at the 5% level as determined by the Duncan Multiple Range Test.

and palmitic acid did not vary in cardiolipin from rats raised on the four diets.

The fatty acid composition of liver cardiolipin found in the present experiment compared favorably with those reported by Getz et al. (1962) for isolated liver mitochondria and microsomes from normal rats. Liver mitochondria had 74.0% of linoleic acid and 12.8% oleic acid while the microsomes showed 57.8% linoleate and 17.0% oleate. Body and Gray (1967) analyzed pig lung cardiolipin and gave a value of 48% for linoleic acid. The presence of eicosatrienoic acid and arachidonic acid has also been reported. This is in contrast to the present experiment where no eicosatrienoic and arachidonic acids were found. This difference may be due to the presence of phosphatidic acid in some of the samples reported and serve as the source of the long chain polyunsaturated fatty acids (Possmayer et al., 1969). Heart cardiolipin from ox has about 80% linoleic acid content and 11.2% oleic acid. It would indicate that heart cardiolipin has a higher reserve of linoleic acid than the liver cardiolipin.

The fatty acid composition of the cardiolipin resembled the tissue triglyceride more than the phospholipids. This is especially evident by the absence of both eicosatrienoic and arachidonic acids which are characteristics of the other phospholipids from EFA

deficient and normal rats respectively. From the standpoint of its composition, cardiolipin may be regarded as a storage phospholipid like the triglycerides and serve as an immediate source of linoleic acid. It has also been shown (de Haas and van Deenen, 1962) that cardiolipin can be acted upon by phospholipase A with the release of the unsaturated fatty acid. The close association of cardiolipin with the other phospholipids may make it a good source of EFA for the other phospholipids.

Hack and Helmy (1967) studying cardiolipin in relation to myocardial infarction suggested that cardiolipin may be degraded and the resulting fatty acids and glycerophosphates may be incorporated into PE and PC and the other phospholipids.

By virtue of the high percentage of cardiolipin in the mitochondrial fraction, it has been connected to the electron transport system, particularly with the cytochrome oxidase. This particular enzyme required phospholipids for activity (Tzagoloff and MacLennon, 1968).

Lysophosphatidylcholine (LPC)

The lyso-derivative of phosphatidylcholine appeared to be the only lysocompound present in the tissues studied. The fatty acid composition of the LPC as shown in Tables 24 and 25 resembled the diacyl phospholipid.

Table 24.--Fatty acid composition[1] of lysophosphatidylcholine from hearts of rats raised on a basal diet containing different levels of linoleic acid.[2]

Fatty Acids[3]	Dietary Linoleate, % of Total Calories in the Diet			
	0.0	0.5	4.0	12.0
16:0	26.0[a][4]	20.7[b]	19.4[b]	21.3[b]
16:1 ω 7	5.8[a]	2.5[a]	trace[5]	1.2[b]
18:0	38.9[a]	36.2[a]	34.8[a]	41.0[a]
18:1 ω 9	19.1[a]	23.0[a]	11.3[b]	7.8[b]
18:2 ω 6	5.9[a]	6.4[a]	16.8[b]	12.1[c]
20:3 ω 9	6.4[a]	4.8[b]	trace	trace
20:4 ω 6	trace[a]	6.4[b]	17.8[c]	16.6[c]
Saturates	64.9	56.9	54.2	62.3
Unsaturates	35.2	43.1	45.9	37.7

[1]Peak area percent.

[2]Second feeding trial.

[3]The first number of the fatty acid designation represents the carbon chain length, and the number following the colon stands for the number of double bonds. The 'ω' number combination indicates the position of the first double bond from the methyl end of the chain.

[4]Means of a given fatty acid followed by the same superscript are not significantly different at the 5% level as determined by the Duncan Multiple Range Test.

[5]Less than 0.1% of total peak area.

Table 25.--Component fatty acids of liver lysophosphatidylcholine from rats fed with a basal diet containing different levels of linoleic acid.[2]

Fatty Acids[3]	Dietary Linoleate, % of Total Calories in the Diet			
	0.0	0.5	4.0	12.0
16:0	24.4[a][4]	22.3[a]	20.2[a]	19.6[a]
16:1 ω 7	5.1[a]	3.5[b]	trace[5]	trace
18:0	27.1[a]	26.3[a]	24.7[a]	23.8[a]
18:1 ω 9	23.5[a]	21.7[a]	19.3[a]	7.1[b]
18:2 ω 6	3.7[a]	5.4[a]	8.5[b]	15.6[c]
20:3 ω 9	13.8[a]	12.2[a]	7.7[a]	trace
20:4 ω 6	2.3[a]	8.7[b]	19.6[c]	34.0[d]
Saturates	51.5	48.6	44.9	43.4
Unsaturates	48.4	51.5	55.1	56.7

[1]Peak area percent.

[2]Second feeding trial.

[3]The first number of the fatty acid designation represents the carbon chain length, and the number following the colon stands for the number of double bonds. The 'ω' number combination indicates the position of the first double bond from the methyl end of the chain.

[4]Means of a given fatty acid followed by the same superscript are not significantly different at the 5% level as determined by the Duncan Multiple Range Test.

[5]Less than 0.1% of total peak area.

There was a correspondingly higher level of linoleic and arachidonic acids in the LPC from both tissues as the level of linoleic acid in the diet increased (between diet D and G) while the eicosatrienoic acid was higher in rats of the deficient state (diet D) than in those rats fed diet G. Compared to the other phospholipids from EFA deficient rats, LPC appeared to be the one to have more linoleic acid than arachidonic acid especially in the heart LPC which had no arachidonic acid. Palmitic acid in heart LPC (Table 25) differed significantly between diet D and diet G. In the liver the level of palmitic acid was progressively lower among the three diets in the direction of higher dietary linoleate (diet D to diet G) although differences were not significant.

The fatty acid composition varied depending upon the source. Leat (1964) analyzed the fatty acid composition of LPC from pig serum and reported that it contained a total of 60.9% for palmitic acid and stearic acid and 25.5% oleic acid. The amounts of arachidonic acid and linoleic acid were 1.6% and 3.9% respectively. The fatty acid composition did not respond to the dietary fatty acids. In the case of the LPC from serum of dogs (Huang and Kuksis, 1967) given corn oil and butter oil, the arachidonic acid appeared to be higher when the animals were fed corn oil than when given butter oil. Linoleic acid content was practically the same under both

diets. The oleic acid in the serum LPC of butterfat fed dogs was higher than in serum of those on a diet containing corn oil. The present study showed that both arachidonic and linoleic acids were affected by the dietary fat as well as palmitic acid. These discrepancies might be due to the differences in the test animal used.

The fatty acids of LPC did not resemble the fatty acid composition found in phosphatidalcholine. This would preclude the plasmalogen as its primary source though some of it may have come from the breakdown of phosphatidalcholine (PC plasmalogen). Although the fatty acids of LPC did not compare with the fatty acid of the lyso derivative of PC produced by phospholipase action, the level of arachidonic acid, particularly in heart PC, may be indicative that part of the LPC found in the tissue might have been derived from PC through metabolism. The absence of arachidonic acid in the heart LPC of EFA deficient rat probably is a result of the ability of the tissue to conserve species of PC containing arachidonic acid. In the presence of linoleic acid in the diet, the tissue may lose the sparing effect of arachidonic acid on the phospholipid molecule.

Sphingomyelin

The fatty acid composition of sphingomyelin in the liver is shown in Table 26. It was characterized by

Table 26.--Fatty acid composition[1] of liver sphingomyelin from rats raised on a basal diet containing different levels of linoleic acid.[2]

Fatty Acid[3]	Dietary Linoleate, % of Total Calories in the Diet			
	0.0	0.5	4.0	12.0
16:0	18.8[4]	14.9	15.8	18.0
16:1 ω 9	5.0	2.3	1.8	1.4
?	trace[5]	1.3	2.2	2.6
18:0	24.9	22.5	21.7	16.4
18:1 ω 9	14.8	15.0	14.4	12.6
18:2 ω 6	4.7	4.4	5.1	10.9
20:1	trace	1.7	4.7	3.5
20:2	10.8	25.3	7.2	2.0
20:4 ω 6	8.6	12.4	16.7	16.6
?	trace	1.4	trace	2.5
?	11.6	9.0	10.4	13.5

[1]Peak area per cent.

[2]Second feeding trial.

[3]The first number of the fatty acid designation represents the carbon chain length and the number following the colon stands for the number of the double bonds. The 'ω' number combination indicates the position of the first double bond from the methyl end of the chain.

[4]Average of two determinations.

the presence of other long chain fatty acids in addition to the normal arachidonic acid or linoleic acid. Arachidonic and linoleic acid content responded to the dietary essential fatty acid. Both acids tended to be high in a diet containing high linoleic acid as in diet F and G and low where the linoleic acid content of the diet is also low (diets D and E). In diet D, palmitoleic acid content was higher than in diet G. Other fatty acids appeared unaffected by the dietary fat.

The fatty acid composition of heart sphingomyelin (Table 27) appeared to be less influenced by dietary fat than that of liver sphingomyelin. The levels of the fatty acids did not vary significantly among the different diets. The component fatty acids found in the present experiment resembled those obtained by Getz et al. (1961) from liver homogenate from normal rats. The primary difference lies in the amount of linoleic acid and arachidonic acid where Getz et al. (1961) reported the amount of 49.6% and 3.04% respectively, whereas in the present experiment only 10.9% and 16.6% were obtained for the same acids.

Leat (1964) studied the sphingomyelin from serum in pigs raised on different fat regimens and found high amounts of saturated fatty acids. He reported about 1% each of both arachidonic and linoleic acid and considerable amounts of long chain fatty acid with more than 20

Table 27.--Component fatty acid[1] found in heart sphingomyelin of rats given different amounts of linoleic acid in the diet.[2]

Fatty Acids[3]	Dietary Linoleate, % of Total Calories in the Diet			
	0.0	0.5	4.0	12.0
16:0	16.5[4]	18.3	14.0	10.5
16:1 ω 7	trace[5]	0.4	1.3	3.9
?	4.0	2.2	3.0	1.7
?	9.6	8.8	8.4	4.7
18:0	20.8	24.8	22.5	25.2
18:1 ω 9	28.6	23.7	30.2	31.7
18:2 ω 6	trace	1.9	1.0	1.3
20:1	3.3	2.2	3.4	2.7
?	3.1	2.0	trace	3.3
?	2.5	4.4	3.6	4.9
20:4 ω 6	11.3	11.1	12.2	10.1

[1]Peak area per cent.

[2]Second feeding trial.

[3]The first number of the fatty acid designation represents the carbon chain length and the number following the colon stands for the number of double bonds. The 'ω' number combination indicates the position of the first double bond from the methyl end of the chain.

[4]Average of two determinations.

[5]Less than 0.1% of total peak area.

carbon atoms. The dietary fat did not show any effect on the fatty acid composition of sphingomyelin. Huang and Kuksis (1963) found the same result in blood serum from dogs raised on butterfat and corn oil as lipid components in the diet. They reported an unusual fatty acid composition characterized by 4.5% palmitic, 25% stearic acid and between 6-7% linoleic acid and the lack of fatty acids with 20-24 carbon atoms.

The differences obtained in the fatty acid composition of sphingomyelin are apparently due to tissue differences. Since the phospholipids used in the present experiment were obtained by two-dimensional chromatography, the chance of contamination with lecithin (Sweeley, 1963) is less likely to occur. It is possible that the turnover of sphingomyelin in the liver may be higher than in the heart to allow for the influence of the dietary fat to be manifested.

Plasmalogen

The plasmalogen content of PE and PC from the rat tissue are shown in Table 28. There was a marked difference in the level of plasmalogen between the two tissues. Whereas liver phospholipid averaged from 4.0% to 8.3%, the heart phospholipids contained from 12.9% to 18.3% plasmalogen. Between the two phospholipid fractions, liver PE had higher plasmalogen content that PC while in

Table 28.--Plasmalogen content[1] of liver and heart phospholipids from rats given a basal diet containing different levels of linoleic acid.[2]

Phospholipid Fraction	Dietary Linoleate, % of Total Calories in the Diet			
	0.0	0.5	4.0	12.0
Liver				
Phosphatidal-ethanolamine	6.1[a3]	8.3[a]	6.2[a]	7.7[a]
Phosphatidal-choline	4.0[a]	5.2[a]	4.4[a]	4.3[a]
Heart				
Phosphatidal-ethanolamine	14.3[a]	13.6[a]	12.9[a]	13.1[a]
Phosphatidal-choline	16.7[a]	18.0[a]	18.3[a]	16.8[a]

[1]Based on phosphorous determination. Percent of total phosphorous recovered.

[2]Second feeding trial.

[3]Means of a given phospholipid followed by the same superscript are not significantly different at the 5% level as determined by the Duncan Multiple Range Test.

the heart tissue there was more plasmalogen in the PC than the PE fraction. The differences in the level of dietary fat did not influence the plasmalogen content of either PE or PC.

The fatty acid composition of plasmalogen as influenced by the dietary fat is shown in Figures 3 and 4. Liver PC from rats raised on a corn oil-containing diet

Figure 3.--Fatty acid composition of phosphatidalethanolamine from heart (A) and liver (B) of rats raised on a basal diet containing different levels of linoleic acid.

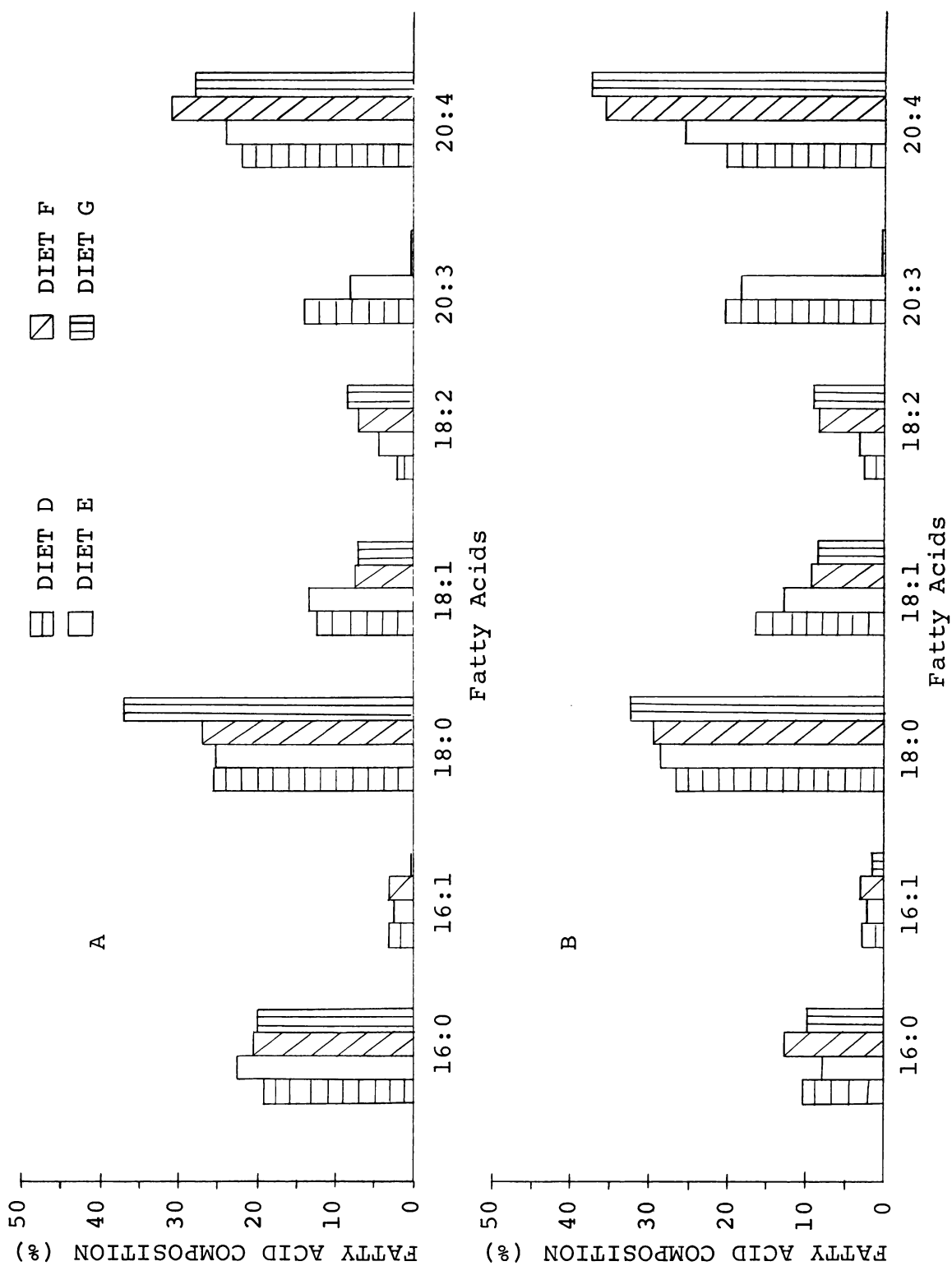
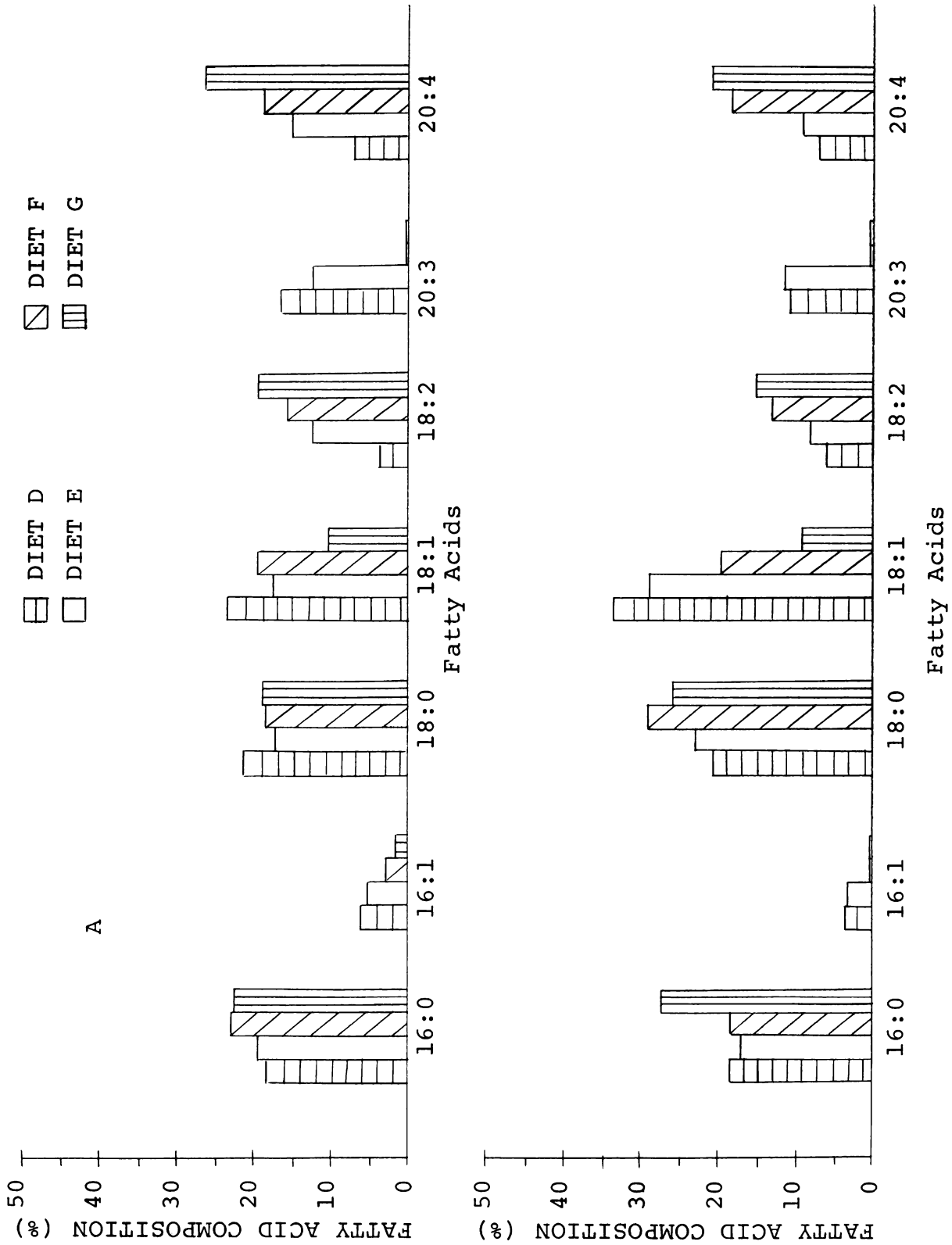

A
B
DIET D
DIET E
DIET F
DIET G
FATTY ACID COMPOSITION (%)
0
10
20
30
40
50
16:0
16:1
18:0
18:1
18:2
20:3
20:4
Fatty Acids

Figure 4.--Fatty acid composition of phosphatidalcholine from heart (A) and liver (B) of rats given a basal diet containing different levels of linoleic acid.


A
DIET D
DIET E
DIET F
DIET G
FATTY ACID COMPOSITION (%)
0
10
20
30
40
50
16:0
16:1
18:0
18:1
18:2
20:3
20:4
Fatty Acids

was characterized by a relatively higher palmitic acid content than stearic acid, while in the PE fraction there was more stearic acid than palmitic acid. At lower levels of dietary linoleate, there was an apparent difference in the ratios of the two saturated fatty acids. These differences in the level of dietary linoleate also resulted in lower amounts of arachidonic acid and linoleic acid and higher contents of both oleic and eicosatrienoic acids.

Heart phosphatidalethanolamine and phosphatidalcholine contained greater amounts of stearic acid than palmitic acid at all levels of dietary linoleate. Neither acid varied with the diet. Both phospholipids exhibited the same variations in oleic and linoleic acid content in relation to the dietary fat composition.

The aldehydes found in the plasmalogens (Tables 29 and 30) were mainly palmitic, stearic and oleic aldehydes. The plasmalogen analogs of both PE and PC in the two tissues exhibited no consistent differences in aldehyde composition with respect to the amount of dietary linoleate.

The level of plasmalogen in the different phospholipid classes appeared unaffected by the diet. Apparently the effect of dietary fat is limited mainly by the fatty acid profile made available to the phospholipid synthesizing enzyme. This was also found in the case of

Table 29.--Fatty aldehyde composition[1] of heart PC and PE plasmalogens from rats raised on a basal diet with different levels of linoleic acid.[2]

Fatty Aldehyde	Dietary Linoleate, % of Total Calories in the Diet			
	0.0	0.5	4.0	12.0
Phosphatidal-ethanolamine				
16:0	30.2[a3]	28.6[a]	26.7[a]	31.3[a]
18:0	28.7[a]	28.1[a]	27.7[a]	27.0[a]
18:1 ω 9	41.1[a]	43.3[a]	45.6[a]	41.2[a]
Phosphatidal-choline				
16:0	42.9[a]	42.7[a]	45.3[a]	44.0[a]
18:0	29.0[a]	23.6[a]	26.8[a]	28.7[a]
18:1 ω 9	27.6[a]	28.7[a]	27.9[a]	27.5[a]

[1]Based on peak area per cent.

[2]Second feeding trial.

[3]Means followed by the same superscript are not significantly different at the 5% level as determined by the Duncan Multiple Range Test.

the fatty acids of the plasmalogen where the changes observed coincided with those observed in the other phospholipid classes. From the fatty acid composition, it would appear that plasmalogen was not synthesized primarily from its endogenous phospholipid analog. It did not resemble the β-position fatty acids found by enzyme

Table 30.--Fatty aldehyde composition[1] of liver phosphatidalcholine and phosphatidalethanolamine from rats fed a basal diet containing different amounts of linoleic acid.[2]

Fatty Aldehyde	Dietary Linoleate, % of Total Calories in the Diet			
	0.0	0.5	4.0	12.0
Phosphatidal-ethanolamine				
16:0	32.5[a3]	31.2[a]	37.9[a]	32.0[a]
18:0	55.7[a]	56.3[a]	46.0[b]	55.1[c]
18:1 ω 9	12.9[a]	12.4[a]	15.9[a]	12.7[a]
Phosphatidal-choline				
16:0	33.2[a]	31.3[a]	27.4[a]	35.1[a]
18:0	32.2[a]	31.3[a]	22.7[a]	30.3[a]
18:1 ω 9	33.4[a]	37.6[a]	49.5[b]	34.5[a]

[1]Based on peak area per cent.

[2]Second feeding trial.

[3]Means followed by the same superscript are not significantly different at the 5% level as determined by the Duncan Multiple Range Test.

hydrolysis considering that the vinyl ether linkage is supposed to be in the α-position of the molecule. It would appear that direct combination of the aldehyde with the lysophospholipid (Bell and White, 1969) may be an important pathway in the synthesis of plasmalogen. The

fatty acid composition of endogenous lysocompound has better resemblance to plasmalogen fatty acid than the β-lyso-derivative of the corresponding diacyl phospholipid.

Plasmalogen or the vinyl ether containing phospholipid have attracted considerable attention not only because of the novel structure but also because of the reactivity attributed to the vinyl ether bond. This in turn has led to speculation on its possible role in the tissues, especially those of the physically active tissues. Heart muscle, for instance, has higher amounts of plasmalogen than in most tissues. This is exemplified by beef heart muscle (MacFarlane and Gray, 1960) which contains more than 50% of PC in the plasmalogen form. At present the exact function of plasmalogen in the animal tissue is still unknown. Studies reported were at most speculative. Rouser and Schloredt (1958) implicated plasmalogen as inhibiting the procoagulant activity of the more unsaturated PE fraction. Roots and Johnston (1968) analyzed fish acclimated at different temperature and indicated that the plasmalogen content of lipid from fish acclimated at 30°C was significantly greater than that of lipid from fish acclimated at 5°C. Thus the replacement of a fatty ester by an α,β-unsaturated ether linkage in the glycerophosphatide could effect changes in properties of the lipid. Differences in the interaction

of phosphatidylcholine and phosphatidalcholine was indicated by the fact that phospholipase A could distinguish between the two molecules (Colacicco and Rapport, 1966).

Enzyme Hydrolysis

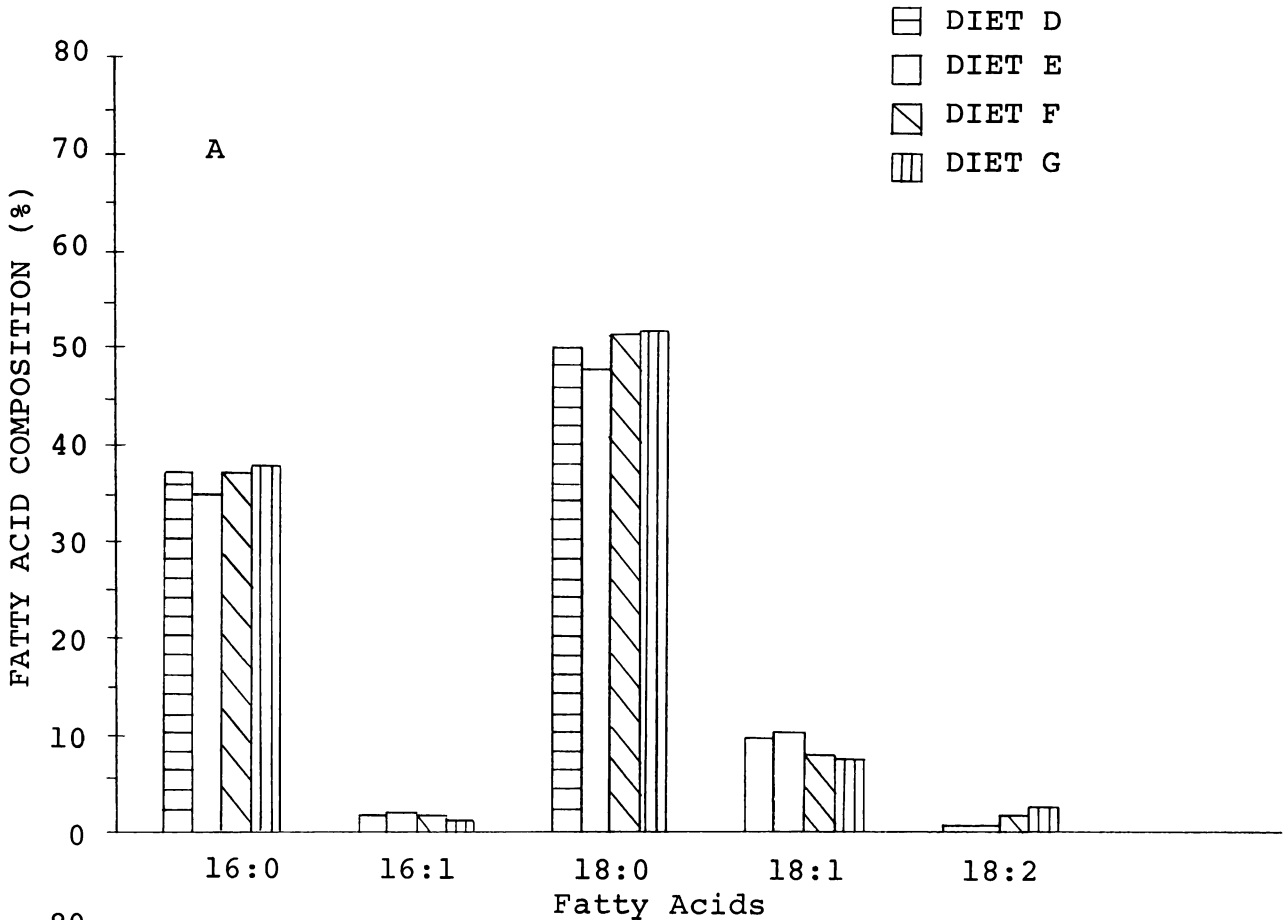
Phosphatidylethanolamine

The fatty acids found in the β-position of liver PE (Figure 5b) were predominantly unsaturated. The saturated fatty acids represented about 10% of the total fatty acid content and consisted mainly of stearic and palmitic acids which remained almost constant with the different diets. In the case of the unsaturated fatty acids, those belonging to the oleic acid family tended to be low with high dietary linoleate while both linoleic and arachidonic acids were high.

The predominant acids in the α-position were palmitic and stearic acids. They represented a constant sum of 89% of the total fatty acids among the four treatments. Palmitoleic acid remained at 1.5% while oleic acid was slightly lower. The highly unsaturated fatty acids were absent especially at lower levels except linoleic acid which appeared at higher amounts of dietary linoleate (Figure 5a).

Heart PE (Figure 6b) had the same fatty acids as those of liver PE in the β-position where much of the

Figure 5.--Fatty acid distribution in α-position (A) and ß-position of liver phosphatidyl-ethanolamine of rats raised on a basal diet containing different levels of dietary linoleate.


DIET D
DIET E
DIET F
DIET G
A
FATTY ACID COMPOSITION (%)
80
70
60
50
40
30
20
10
0
16:0
16:1
18:0
18:1
18:2
Fatty Acids

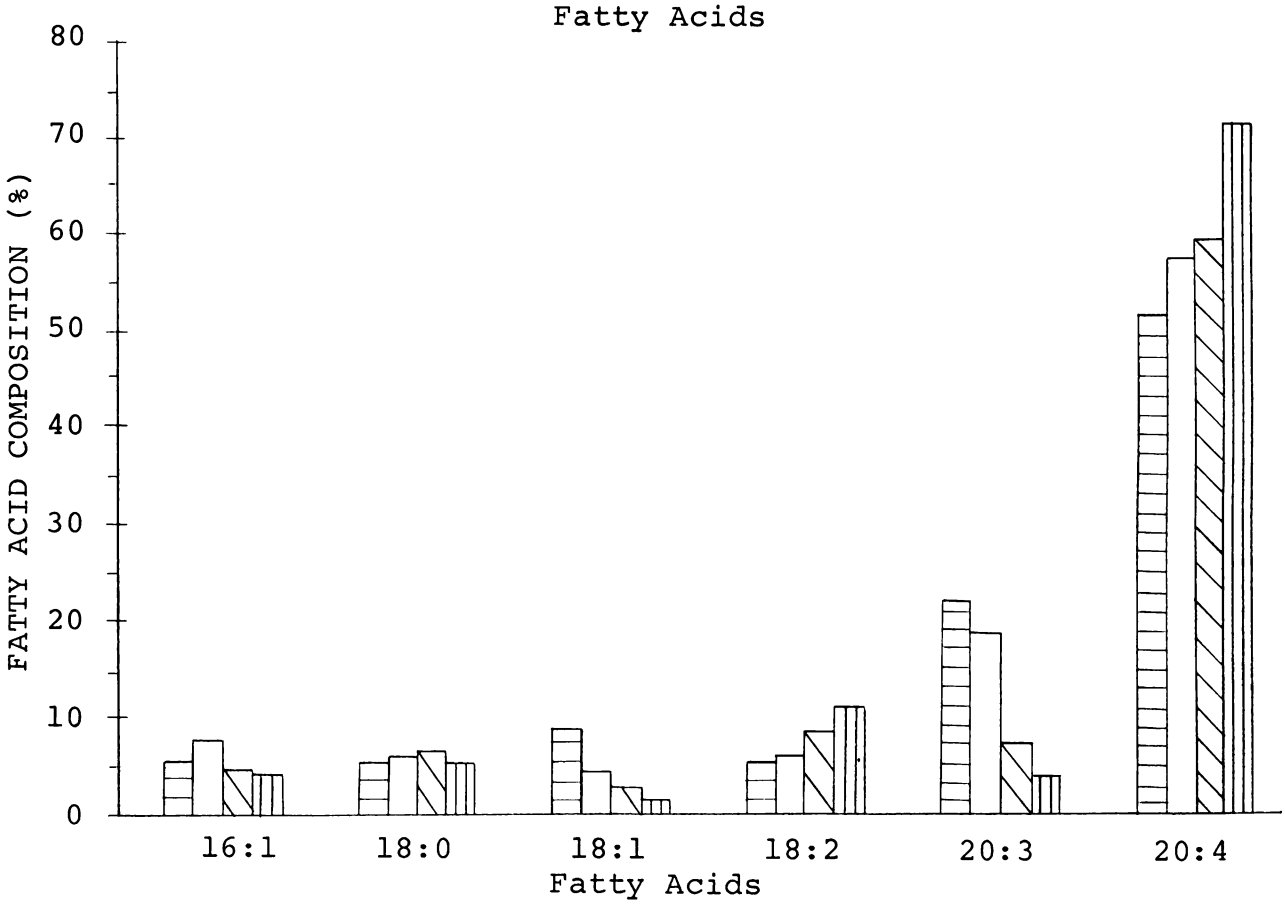
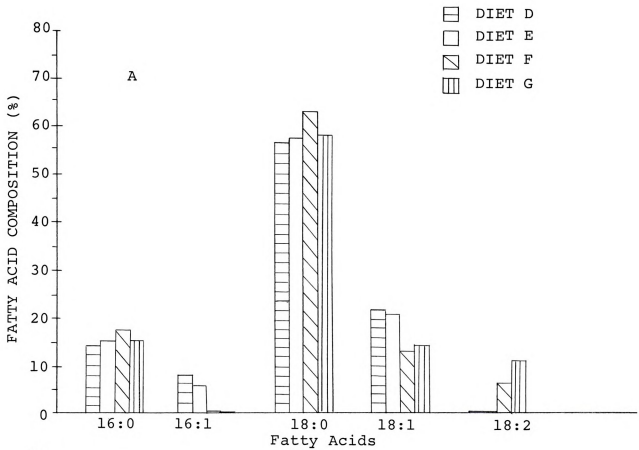
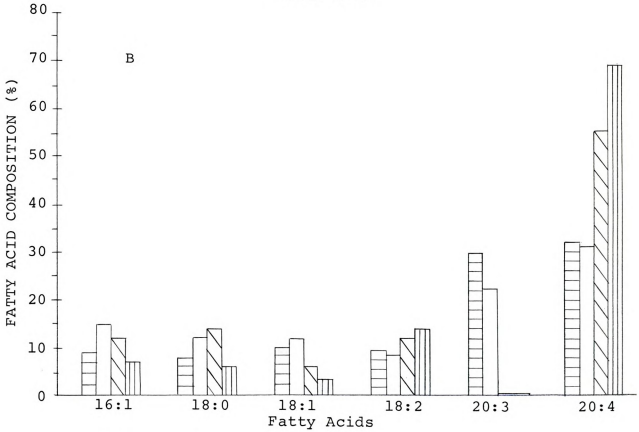

FATTY ACID COMPOSITION (%)
80
70
60
50
40
30
20
10
0
16:1
18:0
18:1
18:2
20:3
20:4
Fatty Acids

Figure 6.--Distribution of fatty acids in the α-position (A) and β-position (B) of heart phosphatidylethanolamine from rats raised on a basal diet containing different levels of dietary linoleate.


DIET D
DIET E
DIET F
DIET G
A
FATTY ACID COMPOSITION (%)
80
70
60
50
40
30
20
10
0
16:0
16:1
18:0
18:1
18:2
Fatty Acids


B
FATTY ACID COMPOSITION (%)
80
70
60
50
40
30
20
10
0
16:1
18:0
18:1
18:2
20:3
20:4
Fatty Acids

differences occurred. Both linoleic and arachidonic acids were high in rat PE whenever the amounts of linoleic acid in the diet was high while eicosatrienoic acid was high in PE of rats fed with hydrogenated coconut oil.

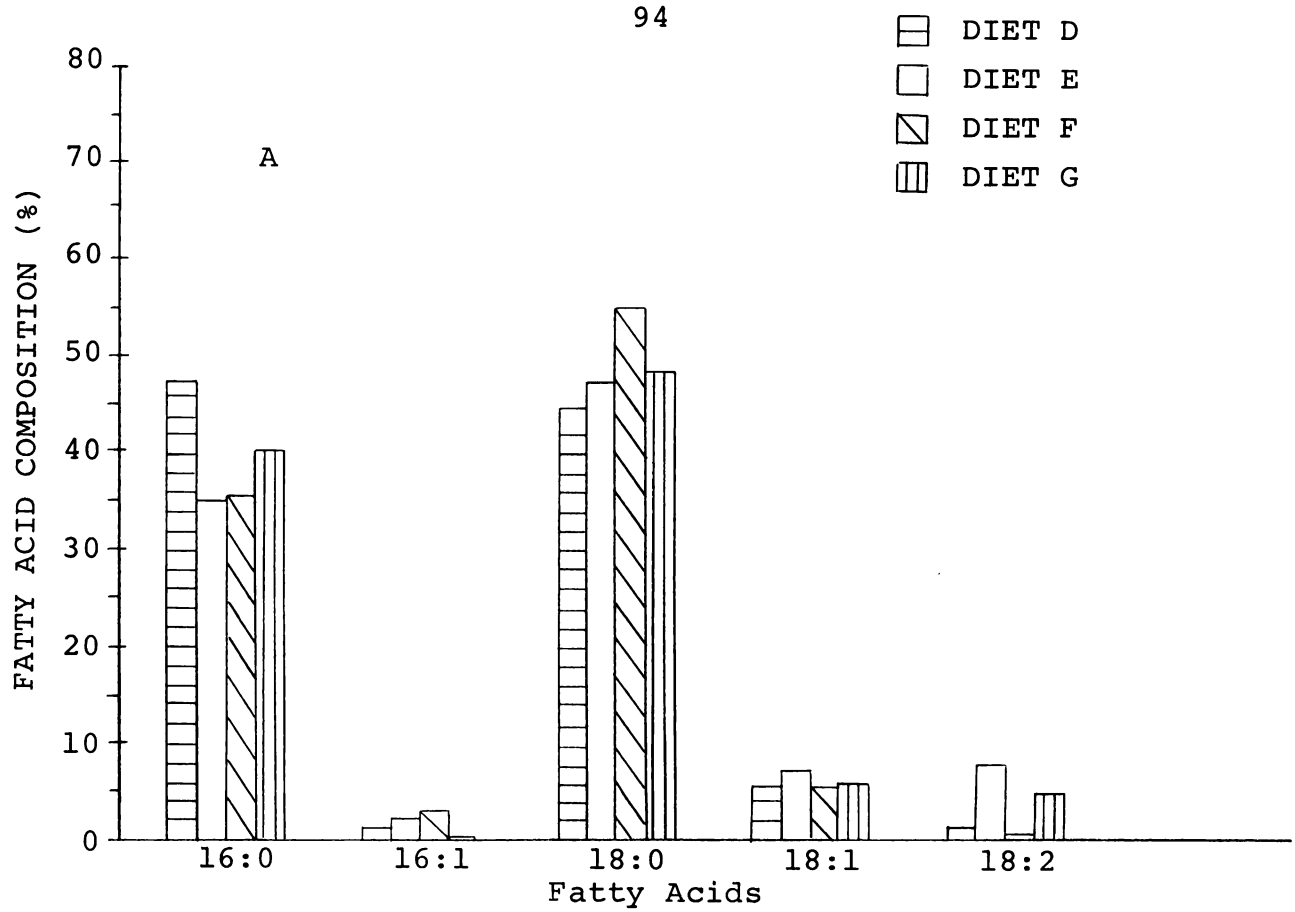
The α-position (Figure 6a) of the PE molecule was occupied mostly by palmitic and stearic acids. These fatty acids remained constant with the diet. Palmitoleic acid varied from 7.9% in diet D to 0.0% in diet G while oleic acid occurred at 21.8% in diet D to 14.3% in diet G. Eicosatrienoic and arachidonic acids were absent in the α-position while linoleic acid appeared only at the highest level of corn oil.

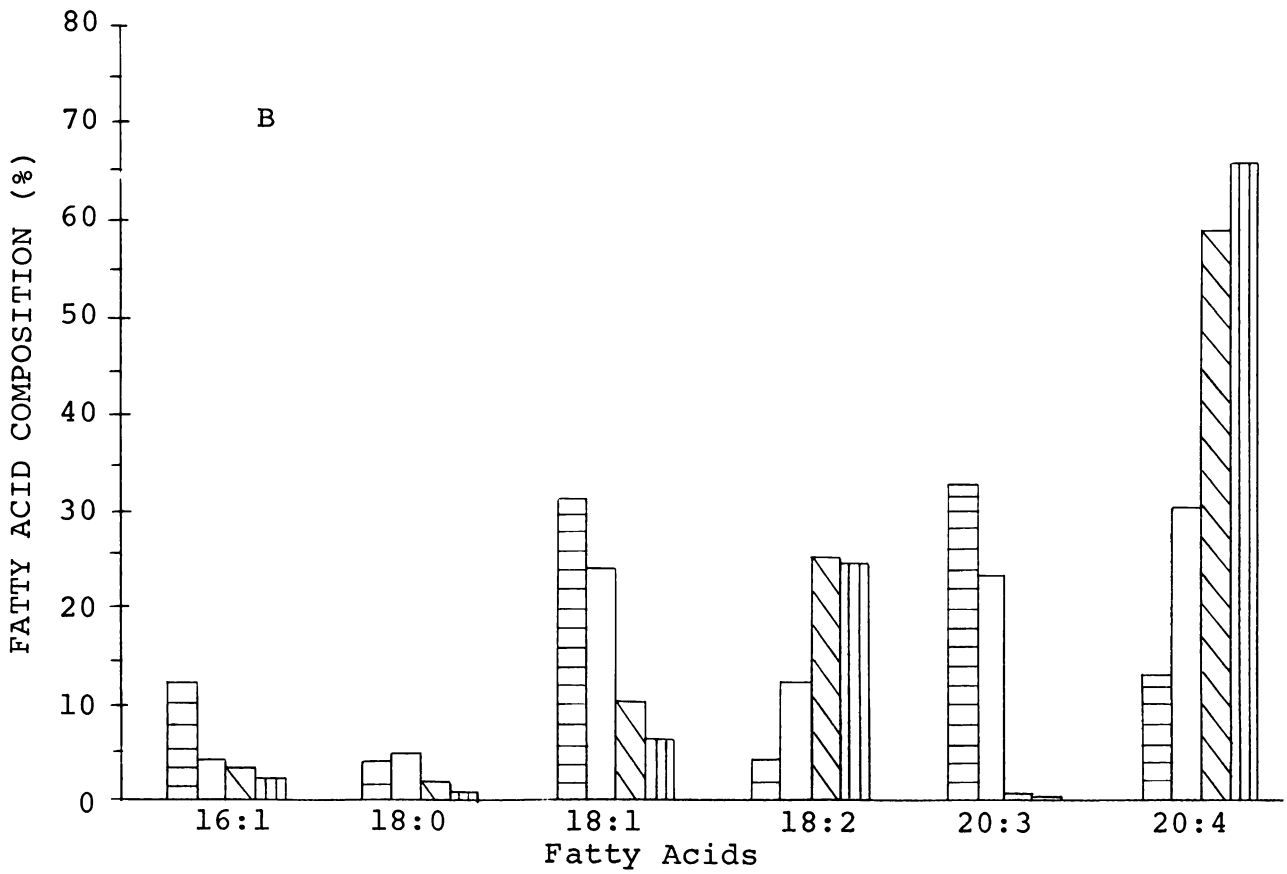
Phosphatidylcholine (PC)

The fatty acids found in the β-position of liver and heart PC were mostly unsaturated while the saturated fatty acids were found in the α-position. The monoenes appeared in both positions of the phospholipid molecule.

The major differences in liver PC (Figure 7) occurred in the β-position where the high levels of linoleic acid in the diet resulted in low levels of eicosatrienoic and palmitoleic acids and high amounts of both arachidonic and linoleic acids. The most noticeable variation occurred among the polyunsaturated fatty acids. The monoenes found in the β-position varied much more than those found in the α-position. A small amount of

Figure 7.--Distribution of the fatty acids in the α-position (A) and β-position (B) of liver phosphatidylcholine from rats fed with a basal diet containing different levels of linoleic acid.


DIET D
DIET E
DIET F
DIET G
A
FATTY ACID COMPOSITION (%)
80
70
60
50
40
30
20
10
0
16:0
16:1
18:0
18:1
18:2
Fatty Acids


B
FATTY ACID COMPOSITION (%)
80
70
60
50
40
30
20
10
0
16:1
18:0
18:1
18:2
20:3
20:4
Fatty Acids

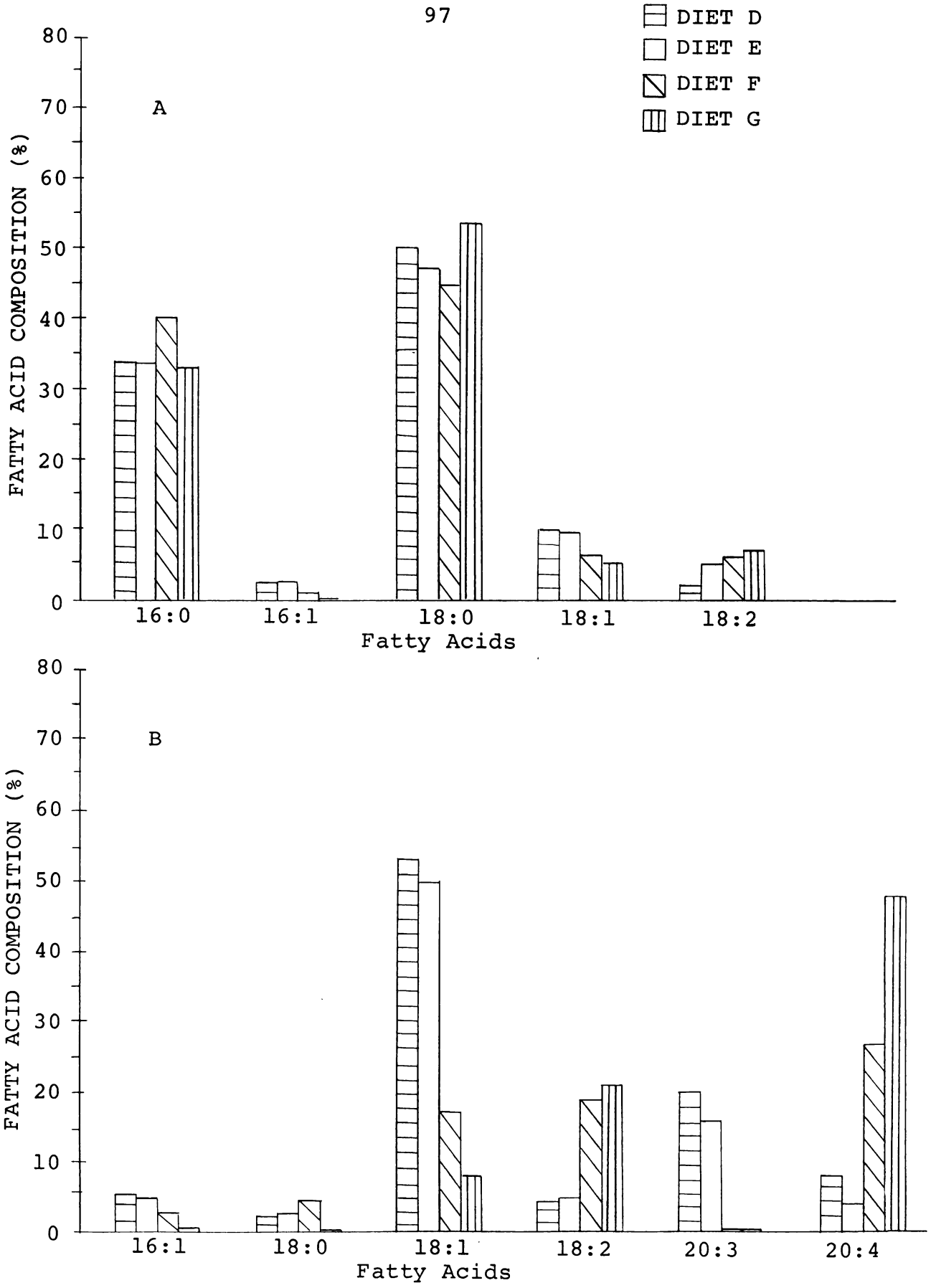
palmitic acid was present in the β-position of liver PC in rats fed diet D which became low with higher levels of dietary linoleate.

Heart PC exhibited essentially the same variation (Figure 8) as found in liver PC. It exhibited elevated amounts of eicosatrienoic acid and oleic acid in EFA deficient rats accompanied by depressed amounts of arachidonic acid and linoleic acid. Meanwhile in the corn oil-fed rat heart PC showed high amounts of linoleic acid group and low amounts of oleic, palmitoleic and eicosatrienoic acids.

The monoenoic fatty acids exhibited larger differences according to diet, in the β-position than in the α-position. The saturated fatty acids in the α-position remained practically constant, while there was an apparent increase of saturated fatty acids particularly palmitic acid in the β-position at high levels of dietary linoleate.

The enzymatic hydrolysis of the two major phospholipid fractions showed both the distribution of the fatty acids and the position where the primary changes took place. The fatty acid distribution in the phospholipid was essentially similar to those already reported (Brenner and Nervi, 1965; Holman and Pudelkewics, 1968; Van Golde et al., 1968). The unsaturated fatty acids were present mostly in the β-position while the bulk of

Figure 8.--Distribution of fatty acids in the α-position (A) and β-position (B) of heart phosphatidylcholine from rats raised on a basal diet containing different amounts of linoleic acid.


DIET D
DIET E
DIET F
DIET G
A
FATTY ACID COMPOSITION (%)
80
70
60
50
40
30
20
10
0
16:0
16:1
18:0
18:1
18:2
Fatty Acids
B
FATTY ACID COMPOSITION (%)
16:1
18:0
18:1
18:2
20:3
20:4
Fatty Acids

the saturated fatty acids were located in the α-position. The monoenes were present in both positions. The fatty acid changes observed by Holman and Pudelkewics (1968) and Van Golde et al. (1968) in rat liver PC were observed on both PC and PE from the liver and the heart of rats. The leveling of the fatty acid content, particularly arachidonic and linoleic acid, in PC above the 1% dietary linoleate (Pudelkewics and Holman, 1968) was not observed in the present experiment from both phospholipids of both tissues but there was a proportional increase that corresponded to the dietary linoleate. Although the feeding time used in the experiment was only one-half of that used by Pudelkewics and Holman (1968), results from other experiments indicated that equilibration of the fatty acid composition is quite rapid (van Golde et al., 1968). This would indicate the influence on the fatty acid composition of the tissue of the other fatty acids present in corn oil (Pfeiffer and Holman, 1962).

The disappearance of oleic and eicosatrienoic acids observed as well as the increase of linolenic and arachidonic acids in the β-position may occur either by direct interchange or by replacement of the whole molecule through synthesis which apparently happens simultaneously in the tissue (Merkle and Lands, 1965).

Blood Lipids

The fatty acid composition of blood plasma PC (Figure 9) from the normal rat resembled the fatty acid composition of total plasma phospholipid reported by Walker (1968). The deficient control, however, showed differences in the levels of stearic and oleic acids while in the present experiment, though not truly comparable, the PC fraction showed equivalent amounts of stearic and oleic acids. Walker (1968) reported 9.4% for stearic acid and 33.9% for oleic acid compared to 22.8% and 23.4% for the respective acids obtained in the present experiment. The discrepancy may be due to the length of time in which the animals were raised on the diet which was about 35 weeks which lead to the accumulation of oleic acid and lowering of stearic acid.

The PE and PC isolated from the red blood cells were analyzed for the fatty acid composition. The PE of blood cells from rats raised on a diet containing corn oil (Figure 10) contained a considerable amount of arachidonic acid (41.4%) and less linoleic acid (12.1%). It was characterized by higher palmitic acid (17.5%) than stearic acid (12.1%), so is the PC fraction where both acids amounted to 38.6% and 16.5% respectively. The linoleic acid fraction of the red cell PC appeared higher than the arachidonic acid content.

Figure 9.--Fatty acid composition of phosphatidylcholine from the red blood cells (A) and blood plasma (B) of rats given a basal diet containing different levels of linoleic acid.

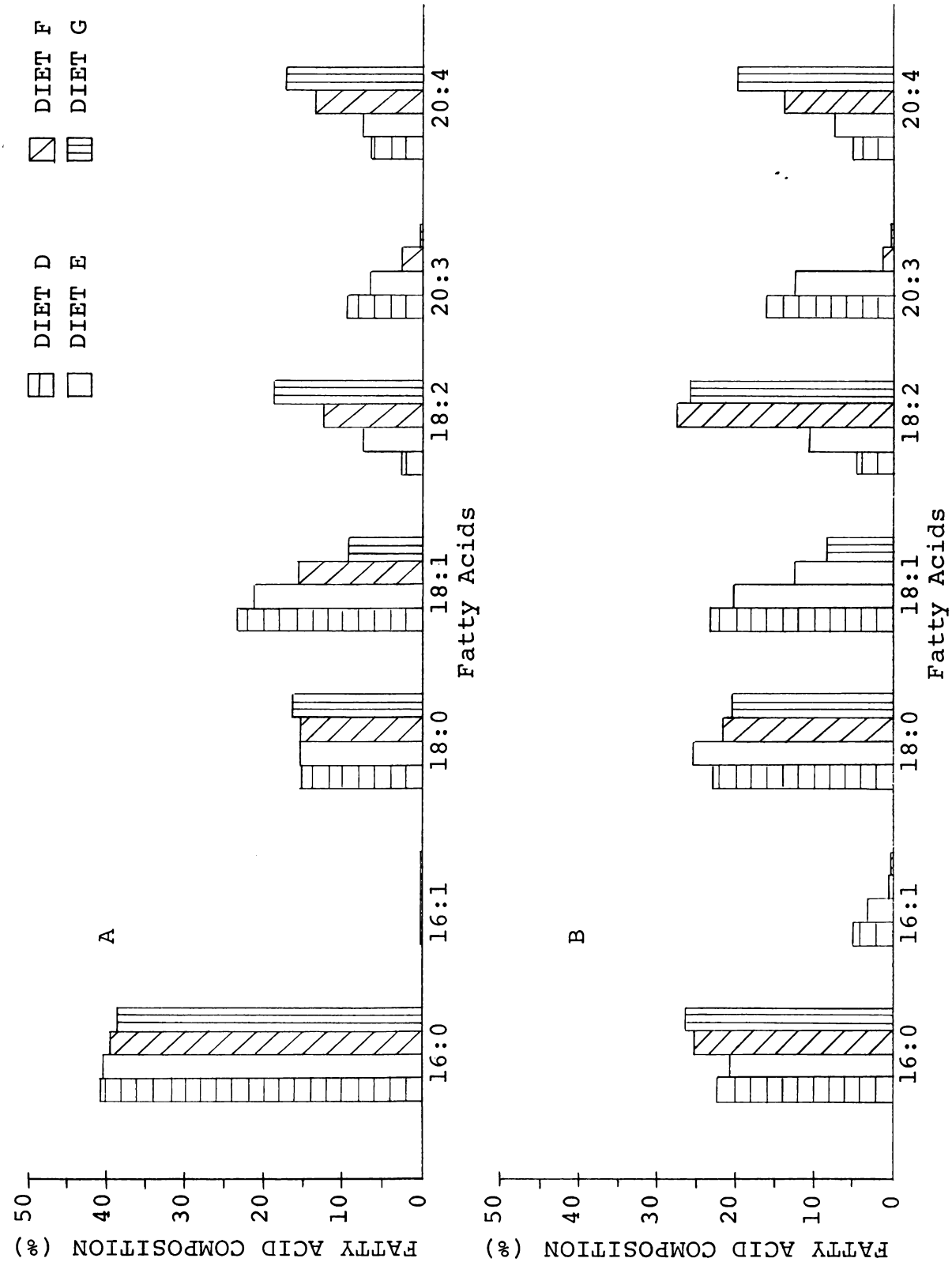
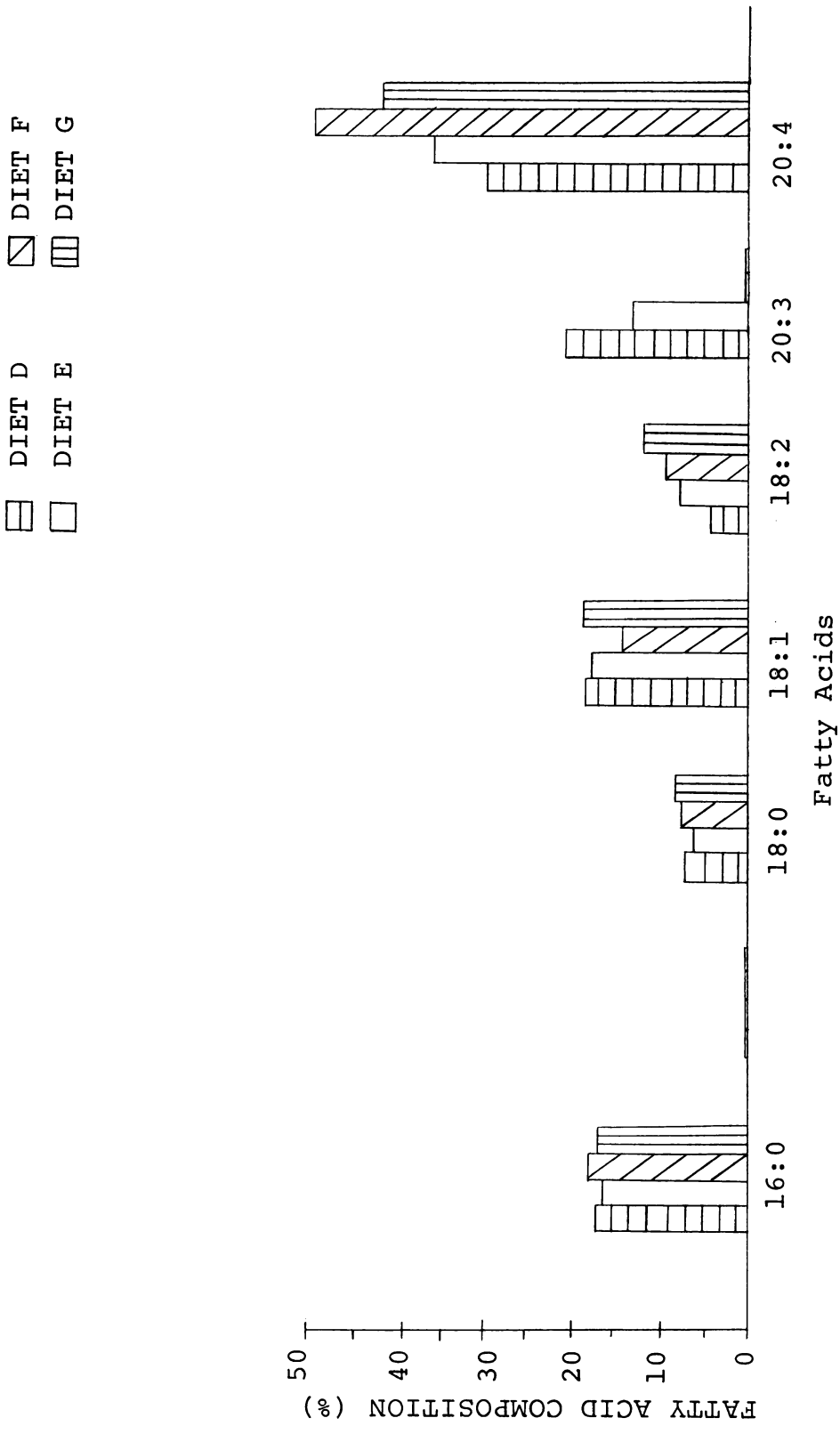

DIET D
DIET E
DIET F
DIET G
A
B
FATTY ACID COMPOSITION (%)
0
10
20
30
40
50
16:0
16:1
18:0
18:1
18:2
20:3
20:4
Fatty Acids

Figure 10.--Fatty acid composition of phosphatidylethanolamine from red blood cells of rats raised on a basal diet containing different levels of linoleic acid.


DIET D
DIET E
DIET F
DIET G
FATTY ACID COMPOSITION (%)
50
40
30
20
10
0
16:0
18:0
18:1
18:2
20:3
20:4
Fatty Acids

The differences in the fatty acids of the oleic and linoleic acid families in the blood follows those observed in the major phospholipids of heart and liver. The use of increased quantities of linoleic acid in the diet was followed by corresponding increase of linoleic and arachidonic acids in the tissues and simultaneous reduction and disappearance of oleic and eicosatrienoic acids. Erythrocyte PE has more pronounced differences in its fatty acid composition than that of the PC fraction.

The four mixtures of dietary fat resulted in differences in the fatty acid pattern of both plasma and red cell lipids which are quite specific being primarily limited to the oleic and linoleic acid families. Administration of coconut oil which contained short chain fatty acids did not lead to incorporation into the tissue phospholipids.

The difference in the fatty acid composition in plasma PC (Figure 9) reflected the variations in other tissue phospholipids. Plasma PC showed also the absence of short chain fatty acids.

The influence of dietary fat on the red cell phospholipid appeared to be exerted mainly in the fatty acid composition rather than the amount or proportion of the phospholipids (Walker and Kummerow, 1963). Feeding with a EFA deficient diet resulted in high proportions

of both oleic and eicosatrienoic acid in both PE and PC of the erythrocyte. Kogle et al. (1961) studied the fatty acid composition of erythrocytes from a number of animal species and reported that the permeability to glycerol and other non-electrolytes was negatively correlated to oleic acid content and positively correlated to the palmitic acid content. Walker and Kummerow (1964) reported that red cells from rats fed with coconut oil were more permeable to glycerol than red cells from corn oil fed rats.

GENERAL DISCUSSION

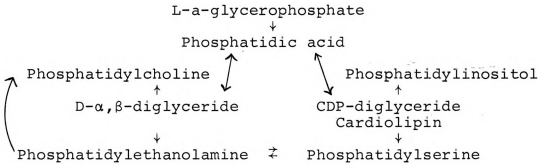
Coconut oil supplied a large amount of medium chain fatty acids in the form of lauric and myristic acids and yet these were not present in the tissue phospholipids studied in the present experiment. It would appear that these medium chain fatty acids underwent elongation and desaturation before being incorporated into the phospholipid molecule. These occurred mostly in the essential fatty acid deficient rats where the linoleic acid was withheld from the diet. The transformations occurred mostly in the formation and incorporation of eicosatrienoic acid, an acid not usually found in the tissue of normal animals. In addition, oleic acid was incorporated in more than the normal level in EFA deficient rats. This shows the ability of the animals to synthesize polyunsaturated fatty acids other than arachidonic acid. These two acids differed from the essential fatty acids, linoleic and arachidonic acids. in the number and position of the double bonds. This apparently is a means by which the animals produce substitute fatty acids to maintain normal cellular organization. The inability of the rat tissue to synthesize essential fatty acids is due to the absence of the

desaturase system that could act on the sixth and ninth carbon atoms to produce linoleic acid which could subsequently be converted to arachidonic acid. The desaturase system found in the rat tissue appeared to act on both palmitic and stearic acids to give the monoenoic acids whose double bonds occur in the ninth carbon atom which could then be converted to the longer and more unsaturated fatty acids.

The presence of linoleic acid in the diet, as in rats given diet G, resulted in the lowering of the levels of both oleic and eicosatrienoic acids in both PE and PC. Eicosatrienoic acid was not present on either phospholipid at the highest level of dietary linoleate used while oleic acid remained at a finite level indicating that the synthesis and incorporation of eicosatrienoic acid was totally inhibited while that of oleic acid was only partially affected.

From the enzymatic studies on the fatty acid distribution in PE and PC, it was established that most of the unsaturated fatty acids were present mainly in the β-position of the phospholipid molecule while the saturated fatty acids were located in the α-position. In turn this reinforced the concept that the changes observed were due to the replacement of oleic and eicosatrienoic acids by linoleic and arachidonic acids. Although the enzyme system showed a great deal of

specificity in positioning the fatty acids in the molecule, one has to account for the differences in the fatty acid composition of a given phospholipid from the two different tissues. This is exemplified by the differences observed in the cardiolipin in the liver and heart lipids where linoleic acid occurred at the levels of 79.0% and 93.3% respectively. Or in the case of PS, those isolated from the heart lipids of normal rats had 12.7% of arachidonic acid and 46.5% linoleic acid while in the liver the same acids amounted to 28.6% and 17.8% respectively. If one were to assume that the following mechanisms were functioning, one has to assume that there are the same number of phosphatidic acid pools as there are phospholipids produced in this pathway in order to account for the differences in the fatty acid composition of the individual phospholipids from different tissues.



The existence of an alternative mechanism for the regulation of the fatty acid composition of phospholipids was found by Lands (1960) when he noted that

liver homogenate has the capacity to acylate lysophosphatidylcholine. Further studies have indicated that this transacylase system is quite specific in directing the fatty acid into the β-position and the saturated fatty acids into the α-position of the phospholipid molecule. The lysocompound could be formed through the action of phospholipases which could act on either α-position (phospholipase A1) or β-position (phospholipase A2) which have been reported to be present in rat liver (Gallai-Hatchard and Thompson, 1965; Marples and Thompson, 1960; Scherphof et al., 1966). Thus it is possible that regulation of the fatty acid incorporation into the lysophospholipid could occur at this stage by means of the side chain which in these cases consisted of the following:

$$-CH_2CH_2N(CH_3)_3 \; ; \; -CH_2CH_2NH_2 \; ; \; -CH_2\underset{NH_3}{CH}COO^-$$

That the side chain influences the reaction of the corresponding phospholipids has been shown by Dawson and Baugham (1963) who reported that the action of Phospholipase A on phospholipid is influenced by the charge of the phospholipid side chain. Thus it would appear that the constitution of the phospholipid is determined not by a single factor but by a large number of factors each one contributing to satisfy the requirement of the individual tissues of the animals.

The differences in the composition of the minor phospholipids are of the same pattern as the phospholipids PE and PC although the levels of fatty acid vary in degree between phospholipids. Cardiolipin, which is present mainly in the mitochondria, possesses a very high level of linoleic acid in rats fed with the high level of dietary linoleate. The mitochondrion is also known to be the locus of phospholipase which is specific for the β-position fatty acid. It may be possible to assume that the incorporation of the linoleic acid could have occurred via the transacylase system through an exchange with oleic acid which is present at high levels in the EFA deficient state. This assumption however is questionable since the cardiolipin present in other tissues appear to possess normal amounts of linoleic acid.

The lysophosphatidylcholines have a fatty acid composition similar to the corresponding diacyl phospholipids suggesting the presence of both isomers of the lysocompound. It is one of the few instances where the fatty acid composition showed that the linoleic acid exceeded the amount of arachidonic acid. If one were to consider the origin of LPC, one may assume that it came from the hydrolytic degradation of PC or was an intermediate for the synthesis of PC (via transacylase system). As an intermediate in the synthesis of PC, it would appear that very little EFA is being incorporated

into the LPC. This is exemplified by the heart LPC where no arachidonic acid appeared in the product from the EFA deficient rats. On the other hand, if one were to consider LPC as a degradation product, it would seem that in heart, at least, some of the arachidonic acid containing species are acted upon by phospholipase A1 to give the β-acyl lysocompound. Whether this is an indication of the conservation of arachidonic acid is questionable. On the other hand, the similarity of the fatty acid composition in terms of the total saturated and unsaturated fatty acids of LPC to that of the plasmalogens may indicate that there is a precursor-product relationship between the two since the vinyl ether bond is known to be labile. The question however arises on the positioning of the fatty acid in the LPC molecule. It has been shown that in the rat tissue, particularly liver, there are two isomers, the α- and the β-acyl LPC. In contrast the plasmalogens are known to have the vinyl ether bond in the α-position of the phospholipid molecule in which case it could only give a β-acyl LPC. Considering the distribution of the fatty acids in both PE and PC, it would mean that the fatty acid present in the plasmalogen must be mostly unsaturated, which is not the case. The only possible explanation of the high amount of saturated fatty acids in plasmalogen would be the non-selective incorporation of the saturated fatty acids in

the β-position of the molecule or if it were synthesized from the lyso-derivative through the direct incorporation of aldehyde, migration of the fatty acid could have occurred from α- to β-position prior to the attachment of the aldehyde.

A similar case appeared in the heart lipids where a high level of unsaturated fatty acids appeared in the PS at the highest level of dietary linoleate. There was an incorporation of about 75% unsaturated fatty acids in the molecule which is more than the value that could be accounted for if the unsaturated fatty acids were to occur solely in the β-position. This non-selective acylation of unsaturated fatty acid was not observed in PS of the liver lipids nor the PI of both heart and liver lipids.

Sphingomyelin showed some tendency to resist changes in its fatty acid composition. Although some variation was observed in the fatty acid composition of liver sphingomyelin, there was almost no change in the sphingomyelin from the heart. Apparently the pathway for the synthesis of sphingomyelin varies from that of the other phospholipids especially in the fatty acid incorporation and renewal. This may also be true in the turn-over of the molecules in the tissue which would account for the very slight change in composition.

SUMMARY

1. Rats were raised for 5 and 6 weeks on a basal diet containing 20% fat as calories with varying amounts of linoleic acid. The weights of the rats were determined at weekly intervals.

2. The animals were killed by decapitation and the blood, heart and liver tissues were isolated for analysis.

3. The lipids were extracted from the tissues using the method of Folch et al. (1957).

4. The different phospholipid classes were separated by means of thin-layer chromatography and the proportions were estimated by determining the phosphorous content.

5. The fatty acid composition of the different phospholipid classes were analyzed by converting the fatty acids into methyl esters and determining the components by gas-liquid chromatography.

6. The plasmalogen contents of both PE and PC were determined along with their fatty acid and aldehyde components.

7. The fatty acid distributions in PE and PC were determined by enzymatic hydrolysis and subsequent isolation, methylation and gas chromatography.

8. The growth rate of rats in the second experiment increased with increasing amount of dietary linoleate. This response was not observed in the first experiment.

9. The varying levels of dietary linoleate did not exert any effect on the proportions of the different phospholipid classes found.

10. The fatty acid composition of both PE and PC showed that high levels of linoleic acid in the diet induced the appearance of high amounts of both arachidonic and linoleic acids in the tissue phospholipids and lesser quantities of oleic and eicosatrienoic acids. At lower levels of dietary linoleate, the levels of linoleic and arachidonic acids were found in the tissue phospholipids while oleic and arachidonic acids were elevated.

11. Minor phospholipids of the phosphatidic acid ester type, PI and PS, exhibited the variations observed in PE and PC with regard to the member fatty acids of the oleic and linoleic acid families. Both PI and PS showed variations in the levels of saturated fatty acids with respect to the diet.

12. Cardiolipin from heart and liver, has a different fatty acid composition from that of other phospholipids. Both eicosatrienoic and arachidonic acids were absent. There was an accumulation of very large amounts of linoleic acid in rats fed the corn oil-containing diet which was partially reduced and apparently replaced by oleic acid in the EFA deficient animals.

13. The lysophospholipid LPC behaved in the same manner as the other phospholipids in its fatty acid composition with respect to the dietary fat, whereby high levels of linoleic acid group and low levels of the oleic acid group were found in the tissue phospholipids when the dietary linoleate was high. High and low levels of the linoleic acid and oleic acid groups respectively existed in the tissue in the absence of dietary linoleate.

14. Sphingomyelin showed a more stable fatty acid composition as very slight variations were observed in the levels of the different fatty acids in response to the dietary fat.

14. Changes in the plasmalogen fraction were primarily in the fatty acid component which exhibited the same changes as the other phospholipids. The levels of plasmalogen in PE and PC did not vary with the diet as well as the aldehyde present.

Suggestions for Further Studies

1. Investigation of conditions that would bring about changes in the composition of the phospholipid may reveal some indication of the role of phospholipids in the tissues.

2. The implication of phospholipids in blood clotting may be studied further in view of the finding that phospholipids are made up of several analogs containing vinyl ether or alkyl ether side chains aside from the differences brought about by variations in fatty acid composition.

3. Use of a model system using phospholipid isolates to study the role of the fatty acid moiety in membrane function of phospholipids.

4. To study the role of natural antioxidants in the tissue in relation to differences in requirement brought about by differences in fatty acid composition.

5. Isolation of the enzyme system responsible for the elongation and desaturation of both the oleic and linoleic acid groups.

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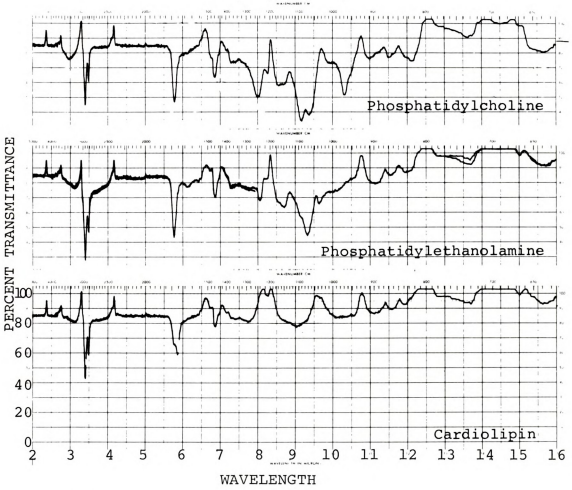
APPENDICES

Appendix A

Reaction of the different classes of phospholipids with different sprays.

Class	Ninhydrin	Molybdate	Dragendorf	NH_4OH-$AgNO_3$
Phosphatidylethanolamine	+	+	-	-
Phosphatidylcholine	-	+	+	-
Phosphatidylserine	+	+	-	-
Phosphatidylinositol	-	+	-	+
Sphingomyelin	-	+	+	-
Cardiolipin	-	+	-	-
Lysophosphatidylcholine	-	+	+	-

Appendix B



IR Spectra in chloroform of phospholipids separated from rat liver by two-dimensional thin-layer chromatography.

Appendix C

Fatty acid composition[1] of heart phosphatidal-choline from rats fed a basal diet containing different levels of linoleic acid.[2]

Fatty Acids[3]	Dietary Linoleate, % of Total Calories in the Diet			
	0.0	0.5	4.0	12.0
16:0	18.4[a4]	17.3[a]	18.6[a]	26.3[b]
16:1 ω 7	3.5[a]	3.2[a]	trace[5]	trace
18:0	20.6[a]	23.5[a]	29.1[b]	27.4[a]
18:1 ω 9	33.7[a]	29.0[b]	18.9[c]	9.2[d]
18:2 ω 6	5.9[a]	8.2[a]	13.0[b]	15.7[b]
20:3 ω 9	10.8[a]	11.6[a]	trace	trace
20:4 ω 6	6.8[a]	9.2[a]	18.4[b]	21.4[b]

[1]Peak area percent.

[2]Second feeding trial.

[3]The first number of the fatty acid designation represents the carbon chain length, and the number following the colon stands for the number of double bonds. The 'ω' number combination indicates the position of the first double bond from the methyl end of the chain.

[4]Means of a given fatty acid followed by the same superscript are not significantly different at the 5% level as determined by the Duncan Multiple Range Test.

[5]Less than 0.1% of total peak area.

Appendix D

Fatty acid composition[1] of liver phosphatidalcholine from rats raised on a basal diet containing different levels of linoleic acid.[2]

Fatty Acids[3]	Dietary Linoleate, % of Total Calories in the Diet			
	0.0	0.5	4.0	12.0
16:0	18.4[a][4]	19.4[b]	23.0[c]	22.6[c]
16:1 ω 7	6.1[a]	5.6[b]	3.0[b]	1.8[b]
18:0	21.5[a]	17.3[a]	18.5[a]	18.9[a]
18:1 ω 9	23.5[a]	17.4[b]	19.5[b]	10.5[c]
18:2 ω 6	4.8[a]	12.7[b]	16.8[c]	19.6[d]
20:3 ω 9	16.7[a]	11.5[b]	trace[5]	trace
20:4 ω 6	7.9[a]	15.1[b]	18.8[b]	26.6[c]

[1]Peak area percent.

[2]Second feeding trial.

[3]The first number of the fatty acid designation represents the carbon chain length, and the number following the colon stands for the number of double bonds. The 'ω' number combination indicates the position of the first double bond from the methyl end of the chain.

[4]Means of a given fatty acid followed by the same superscript are not significantly different at the 5% level as determined by the Duncan Multiple Range Test.

[5]Less than 0.1% of total peak area.

Appendix E

Fatty acid composition[1] of heart phosphatidal-ethanolamine from rats raised on a basal diet containing different levels of linoleic acid.[2]

Fatty Acids[3]	Dietary Linoleate, % of Total Calories in the Diet			
	0.0	0.5	4.0	12.0
16:0	10.6[a4]	7.9[a]	12.8[a]	9.9[a]
16:1 ω 7	2.8[a]	2.3[a]	3.2[a]	1.9[a]
18:0	26.8[a]	28.6[a]	29.5[a]	32.4[a]
18:1 ω 9	16.5[a]	12.8[b]	9.3[c]	8.4[c]
18:2 ω 6	2.5[a]	3.2[a]	8.6[b]	9.2[b]
20:3 ω 9	20.6[a]	18.5[a]	trace[5]	trace
20:4 ω 6	20.6[a]	25.8[b]	36.6[c]	38.4[c]

[1]Peak area percent.

[2]Second feeding trial.

[3]The first number of the fatty acid designation represents the carbon chain length, and the number following the colon stands for the number of double bonds. The 'ω' number combination indicates the position of the first double bond from the methyl end of the chain.

[4]Means of a given fatty acid followed by the same superscript are not significantly different at the 5% level as determined by the Duncan Multiple Range Test.

[5]Less than 0.1% of total peak area.

Appendix F

Fatty acid composition[1] of liver phosphatidal-ethanolamine from rats raised on a basal diet containing different levels of linoleic acid.[2]

Fatty Acids[3]	Dietary Linoleate, % of Total Calories in the Diet			
	0.0	0.5	4.0	12.0
16:0	19.1[a4]	22.4[a]	22.4[a]	19.9[a]
16:1 ω 7	2.9[a]	2.3[a]	2.8[a]	trace[5]
18:0	25.5[a]	25.3[a]	27.8[a]	37.1[b]
18:1 ω 9	14.5[a]	13.4[a]	8.5[b]	7.1[b]
18:2 ω 6	2.1[a]	4.6[a]	7.0[b]	8.5[b]
20:3 ω 9	14.1[a]	8.2[b]	trace	trace
20:4 ω 6	21.9[a]	23.9[a]	31.0[b]	28.0[b]
Saturates	44.6	47.7	48.4	57.0
Unsaturates	53.5	52.4	48.3	43.6

[1]Peak area percent.

[2]Second feeding trial.

[3]The first number of the fatty acid designation represents the carbon chain length, and the number following the colon stands for the number of double bonds. The 'ω' number combination indicates the position of the first double bond from the methyl end of the chain.

[4]Means of a given fatty acid followed by the same superscript are not significantly different at the 5% level as determined by the Duncan Multiple Range Test.

[5]Less than 0.1% of total peak area.

Appendix G

Fatty acids[1] found in the α-position of liver phosphatidylethanolamine from rats raised on a basal diet containing different levels of linoleic acid.[2]

Fatty Acids[3]	Dietary Linoleate, % of Total Calories in the Diet			
	0.0	0.5	4.0	12.0
16:0	37.0[4]	34.8	37.0	37.7
16:1 ω 7	1.5	1.8	1.5	1.2
18:0	50.0	52.5	51.1	51.4
18:1 ω 9	9.3	10.5	7.8	7.4
18:2 ω 6	0.0	0.0	1.6	2.3
20:4 ω 6	1.2	0.0	0.0	0.0

[1]Peak area percent.

[2]Second feeding trial.

[3]The first number of the fatty acid designation represents the carbon chain length, and the number following the colon stands for the number of double bonds. The 'ω' number combination indicates the position of the first double bond from the methyl end of the chain.

[4]Average of two determinations.

Appendix H

Fatty acids[1] found in the β-position of liver phosphatidylethanolamine from rats fed with a basal diet containing different levels of linoleic acid.[2]

Fatty Acids[3]	Dietary Linoleate, % of Total Calories in the Diet			
	0.0	0.5	4.0	12.0
16:1 ω 7	5.5[4]	7.0	5.6	4.1
18:0	5.1	5.9	6.6	5.2
18:1 ω 9	8.7	4.6	2.9	1.5
18:2 ω 6	5.5	6.0	8.3	11.0
20:3 ω 9	23.7	18.7	7.2	3.7
20:4 ω 6	51.5	57.8	69.2	74.5

[1]Peak area percent.

[2]Second feeding trial.

[3]The first number of the fatty acid designation represents the carbon chain length, and the number following the colon stands for the number of double bonds. The 'ω' number combination indicates the position of the first double bond from the methyl end of the chain.

[4]Average of two determinations.

Appendix I

Fatty acids[1] found in the α-position of heart phosphatidylethanolamine from rats raised on a basal diet containing different levels of linoleic acid.[2]

Fatty Acids[3]	Dietary Linoleate, % of Total Calories in the Diet			
	0.0	0.5	4.0	12.0
16:0	13.9[4]	15.0	17.3	15.1
16:1 ω 7	7.9	5.9	trace[5]	trace
18:0	56.4	57.2	63.1	58.1
18:1 ω 9	21.8	20.8	12.9	14.3
18:2 ω 6	0.0	0.0	6.7	12.4

[1]Peak area percent.

[2]Second feeding trial.

[3]The first number of the fatty acid designation represents the carbon chain length, and the number following the colon stands for the number of double bonds. The 'ω' number combination indicates the position of the first double bond from the methyl end of the chain.

[4]Less than 0.1% total peak area.

[5]Average of two determinations.

Appendix J

Fatty acid composition[1] of β-position of heart phosphatidylethanolamine from rats raised on a basal diet containing different levels of linoleic acid.[2]

Fatty Acids[3]	Dietary Linoleate, % of Total Calories in the Diet			
	0.0	0.5	4.0	12.0
16:1 ω 7	9.0[4]	14.9	12.2	6.9
18:0	8.8	12.6	14.1	6.0
18:1 ω 9	10.2	12.1	6.1	3.8
18:2 ω 6	9.8	8.7	12.2	14.4
20:3 ω 9	30.0	22.5	trace[5]	trace
20:4 ω 6	32.2	29.1	55.3	68.9

[1]Peak area percent.

[2]Second feeding trial.

[3]The first number of the fatty acid designation represents the carbon chain length, and the number following the colon stands for the number of double bonds. The 'ω' number combination indicates the position of the first double bond from the methyl end of the chain.

[4]Average of two determinations.

[5]Less than 0.1% total peak area.

Appendix K

Fatty acid[1] found in the ß-position of liver phosphatidylcholine from rats raised on a basal diet containing different levels of linoleic acid.[2]

Fatty Acids[3]	Dietary Linoleate, % of Total Calories in the Diet			
	0.0	0.5	4.0	12.0
16:0	12.3[4]	4.2	3.6	2.6
16:1 ω 7	4.6	5.1	2.2	1.5
18:0	1.2	trace[5]	trace	trace
18:1 ω 9	31.6	24.0	10.3	6.6
18:2 ω 6	4.5	12.3	25.4	23.7
20:3 ω 9	32.8	23.6	trace	trace
20:4 ω 6	13.1	30.3	58.9	65.8

[1]Peak area percent.

[2]Second feeding trial.

[3]The first number of the fatty acid designation represents the carbon chain length, and the number following the colon stands for the number of double bonds. The 'ω' number combination indicates the position of the first double bond from the methyl end of the chain.

[4]Average of two determinations.

[5]Less than 0.1% of total peak area.

Appendix L

Fatty acids[1] found in the α-position of liver phosphatidylcholine from rats raised on a basal diet containing different levels of linoleic acid.[2]

Fatty Acids[3]	Dietary Linoleate, % of Total Calories in the Diet			
	0.0	0.5	4.0	12.0
16:0	47.3[4]	34.8	35.6	40.6
16:1 ω 7	1.4	2.5	3.3	trace[5]
18:0	44.4	47.2	55.5	48.4
18:1 ω 9	5.8	7.5	5.6	6.0
18:2 ω 6	1.4	8.1	trace	5.1

[1]Peak area percent.

[2]Second feeding trial.

[3]The first number of the fatty acid designation represents the carbon chain length, and the number following the colon stands for the number of double bonds. The 'ω' number combination indicates the position of the first double bond from the methyl end of the chain.

[4]Average of two determinations.

[5]Less than 0.1% of total peak area.

Appendix M

Fatty acid composition[1] in the β-position of phosphatidylcholine from hearts of rats raised on a basal diet containing different levels of linoleic acid.[2]

Fatty Acids[3]	Dietary Linoleate, % of Total Calories in the Diet			
	0.0	0.5	4.0	12.0
16:0	6.1[4]	16.7	28.0	23.4
16:1 ω 7	5.4	5.0	2.9	trace[5]
18:0	2.4	3.0	4.9	trace
18:1 ω 9	53.6	50.9	17.6	8.2
18:2 ω 6	3.8	4.2	19.8	20.5
20:3 ω 9	20.3	16.0	trace	trace
20:4 ω 6	8.4	4.3	26.9	48.0

[1]Peak area percent.

[2]Second feeding trial.

[3]The first number of the fatty acid designation represents the carbon chain length, and the number following the colon stands for the number of double bonds. The 'ω' number combination indicates the position of the first double bond from the methyl end of the chain.

[4]Average of two determinations.

[5]Less than 0.1% of total peak area.

Appendix N

Fatty acids found[1] in the α-position of heart phosphatidylcholine from rats raised on a basal diet containing different levels of linoleic acid.[2]

Fatty Acids[3]	Dietary Linoleate, % of Total Calories in the Diet			
	0.0	0.5	4.0	12.0
16:0	34.2[4]	34.4	40.7	33.1
16:1 ω 7	2.9	2.8	1.2	trace[5]
18:0	50.3	47.7	45.1	53.9
18:1 ω 9	10.2	9.8	6.7	5.5
18:2 ω 6	2.4	5.3	6.5	7.4

[1]Peak area percent.

[2]Second feeding trial.

[3]The first number of the fatty acid designation represents the carbon chain length, and the number following the colon stands for the number of double bonds. The 'ω' number combination indicates the position of the first double bond from the methyl end of the chain.

[4]Average of two determinations.

[5]Less than 0.1% of total peak area.

Appendix O

Fatty acid composition[1] of phosphatidylethanolamine from red blood cells of rats raised on a basal diet containing different levels of dietary linoleate.[2]

Fatty Acids[3]	Dietary Linoleate, % of Total Calories in the Diet			
	0.0	0.5	4.0	12.0
16:0	17.3[a4]	16.9[a]	18.6[a]	17.3[a]
16:1 ω 7	trace[5]	trace	trace	trace
18:0	7.6[a]	6.4[a]	7.9[a]	8.8[a]
18:1 ω 9	18.8[a]	18.2[a]	14.3[b]	19.1[c]
18:2 ω 6	4.5[a]	7.9[b]	9.4[b]	12.2[c]
20:3 ω 6	21.3[a]	12.6[b]	trace	trace
20:4 ω 6	29.7[a]	35.8[b]	49.6[c]	42.4[d]

[1]Peak area percent.

[2]Second feeding trial.

[3]The first number of the fatty acid designation represents the carbon chain length, and the number following the colon stands for the number of double bonds. The 'ω' number combination indicates the position of the first double bond from the methyl end of the chain.

[4]Means of a given fatty acid followed by the same superscript are not significantly different at the 5% level as determined by the Duncan Multiple Range Test.

[5]Less than 0.1% of total peak area.

Appendix P

Fatty acid composition[1] of phosphatidylcholine from red blood cells of rats raised on a basal diet containing different amounts of linoleic acid.[2]

Fatty Acids[3]	Dietary Linoleate, % of Total Calories in the Diet			
	0.0	0.5	4.0	12.0
16:0	41.9[a][4]	40.6[a]	39.5[a]	38.6[a]
16:1 ω 7	trace[5]	trace	3.7[a]	1.8[a]
18:0	15.6[a]	15.4[a]	14.4[a]	15.5[a]
18:1 ω 9	23.4[a]	21.3[a]	15.4[a]	9.3[b]
18:2 ω 6	2.9[a]	7.6[b]	12.5[c]	18.9[d]
20:3 ω 9	9.6[a]	6.9[a]	2.5[b]	trace
20:4 ω 6	6.5[a]	7.7[a]	12.3[b]	15.6[b]

[1]Peak area percent.

[2]Second feeding trial.

[3]The first number of the fatty acid designation represents the carbon chain length, and the number following the colon stands for the number of double bonds. The 'ω' number combination indicates the position of the first double bond from the methyl end of the chain.

[4]Means of a given fatty acid followed by the same superscript are not significantly different at the 5% level as determined by the Duncan Multiple Range Test.

[5]Less than 0.1% of total peak area.

Appendix Q

Fatty acid composition[1] of blood plasma phosphatidylcholine from rats fed a basal diet containing different levels of linoleic acid.[2]

Fatty Acids[3]	Dietary Linoleate, % of Total Calories in the Diet			
	0.0	0.5	4.0	12.0
16:0	22.2[a][4]	20.7[a]	25.1[b]	26.4[b]
16:1 ω 7	4.8[a]	3.0[a]	trace[5]	trace
18:0	23.8[a]	25.5[a]	21.6[a]	20.3[a]
18:1 ω 9	23.4[a]	20.3[a]	12.8[b]	8.2[c]
18:2 ω 6	4.5[a]	10.3[b]	26.1[c]	25.8[c]
20:3 ω 9	16.2[a]	12.6[b]	1.2[c]	trace
20:4 ω 6	5.1[a]	7.6[a]	13.8[b]	19.7[c]

[1]Peak area percent.

[2]Second feeding trial.

[3]The first number of the fatty acid designation represents the carbon chain length, and the number following the colon stands for the number of double bonds. The 'ω' number combination indicates the position of the first double bond from the methyl end of the chain.

[4]Means of a given fatty acid followed by the same superscript are not significantly different at the 5% level as determined by the Duncan Multiple Range Test.

[5]Less than 0.1% of total peak area.