

The Essential Fatty Acids

MICHAEL GUARNIERI

*Department of Physiological Chemistry, The Johns Hopkins University
School of Medicine, Baltimore, Maryland*

AND

RALPH M. JOHNSON

College of Science, Utah State University, Logan, Utah

I.	Introduction	115
II.	Nomenclature	116
III.	Structural Requirements	118
	A. Determination of Essential Fatty Acids Activity	118
	B. Possible Endogenous Precursors	120
	C. Trans Fatty Acids	120
	D. Positional Isomerism	123
	E. Essential Fatty Acid Derivatives	127
IV.	Metabolism and Function of Polyenoic Acids	128
	A. Polyenoic Acid Interactions	128
	B. Biosynthesis	132
	C. Phospholipid Metabolism	133
	D. Comparative Metabolism of Linoleate and Arachidonate	137
	E. Essential Fatty Acids and Vitamins	138
	F. Essential Fatty Acids and Hormones	139
	G. Polyenoic Acids and Cholesterol Esters	141
	H. Prostaglandins	142
	I. Essential Fatty Acid Deficiency Lesions	144
	J. Essential Fatty Acid Metabolites: Lipid Peroxidation ..	145
	K. Diet and Tissue Polyenoic Acid Content	148
V.	Essential Fatty Acids and Membrane Function	161
VI.	Summary	166
	References	167

I. Introduction

Rats reared on diets deficient in essential fatty acids (EFA) cease to gain weight at approximately 3 months, become infertile, develop dermal lesions, and have an increased water uptake compared to rats reared on normal diets. Unlike an essential amino acid deficiency, EFA deficiency does not cause death to the animal. At the cellular level, the phospholipids in EFA-deficient animals have a high content

of polyenoic acids derived from oleic and palmitoleic acids, whereas phospholipids in animals fed normal diets contain exclusively polyenoic acids of the linoleic and linolenic acid family. Prostaglandins are formed from EFA, but EFA deficiency symptoms cannot be cured by oral or intravenous administration of prostaglandins. Membranous structures, mitochondria, lysosomes, erythrocytes—have a high polyenoic acid content. These structures isolated from rats fed EFA-deficient diet are similar to those isolated from rats fed normal diets. Only when the mitochondria, lysosomes, or erythrocytes are stressed—for example, aging, phospholipase treatment, dispersion in hypotonic solutions—do differences between the structures isolated from normal and EFA-deficient animals become apparent. There is no deficiency symptom that is not reversed, almost immediately, by feeding EFA. Overall, as long as EFA-deficient animals are not stressed, they do not appear greatly different from normal animals. Burr and Burr demonstrated in 1929 the nutritional necessity for EFA. To this date, despite efforts by some of the most gifted and determined biochemical nutritionists, it is not known why EFA are dietary requirements.

Recent reviews on EFA have been made by Sinclair (1958, 1964) and Aaes Jorgensen (1961, 1966). Klenk (1965a, 1965b) has reviewed the metabolism of polyenoic acids. Carroll (1965) has reviewed the influence of dietary fat on the fatty acid composition of tissue lipids. Rothblat and Kritchevsky (1967) have edited a recent symposium on lipid metabolism in tissue culture cells. Bloch (1969) has reviewed the enzyme synthesis of monounsaturated fatty acids. Polyenoic acid chemistry has been reviewed by Holman (1966) and others.

This manuscript was with the editors when a review of the 1961-1967 EFA literature by Alfin-Slater and Aftergood (1968) was published. We have taken advantage of this excellent review by deleting historical information and by adding speculative material, more than otherwise would be possible. Particular attention has been paid to lipoxide, peroxide, and prostaglandin polyenoic acid reactions. The reactions are similar in mechanism. Since lipoxidation and prostaglandin formation are the only enzyme-catalyzed reactions known to require substrates having an EFA-type structure, microsomal lipid peroxidation may somehow be involved in the EFA problem.

II. Nomenclature

Common names for some fatty acids have become entrenched in the literature. These are listed below with their chemical names.

<u>Chemical name</u>	<u>Common name</u>
Dodecanoic	Lauric
Tetradecanoic	Myristic
Hexadecanoic	Palmitic
<i>cis</i> -9-Hexadecanoic	Palmitoleic
Octadecanoic	Stearic
<i>cis</i> -9-Octadecanoic	Oleic
<i>trans</i> -9-Octadecanoic	Elaidic
<i>cis</i> -11-Octadecanoic	Vaccinic
<i>cis</i> -9, <i>cis</i> -12-Octadecadienoic	Linoleic
<i>cis</i> -9, <i>cis</i> -12, <i>cis</i> -15-Octadecatrienoic	Linolenic
<i>cis</i> -5, <i>cis</i> -8, <i>cis</i> -11, <i>cis</i> -14-Eicosatetraenoic	Arachidonic

The use of abbreviated formulas has become common (cf. Holman, 1966). The two most common ones list the position of the double bonds followed by a number indicating the carbon chain length. Linoleic acid is written $\Delta^{9:10, 12:13}\text{-C}_{18}$ or 9,12-18:2. In the latter case the number of double bonds is listed following a colon after the number indicating the chain length. Since the number of bonds has been specified the second listing is redundant, but it serves to introduce a useful shorthand notation.

Fatty acids are lengthened by the addition of two carbons at the carboxyl end. Desaturation occurs between the carboxyl group and the double bond nearest the carboxyl group. The number of carbons between the methyl end of the acid and the double bond nearest the methyl end is fixed. Since a divinyl methane rhythm (-CH₂-CH=CH-) is maintained in desaturation reactions, the position of all double bonds relative to the methyl end of the molecule is fixed. Therefore, the exact structure of any polyenoic acid can be given by three numbers having the following values: length of carbon chain; number of double bonds; number of carbons between terminal double bond and methyl group, or the omega (ω) carbon. Linoleic acid becomes 18:2 ω 6. Oleic acid is 18:1 ω 9.

The value of this shorthand notation exceeds its brevity. The omega nomenclature immediately indicates that all unsaturated fatty acids belong to groups dependent on the distance of the terminal double bond from the methyl end of the molecule. Once a group or family is specified, all members of that family are known, because there are no interconversions of groups. There are four common families of unsaturated fatty acids in animals. Their characteristics and major constituents are briefly introduced here.

Palmitoleic, $\omega 7$	(9-)16:1 $\omega 7$ (11-)18:1 $\omega 7$	Elevated in EFA deficiency
Oleic, $\omega 9$	(9-)18:1 $\omega 9$ (5,8,11-)20:3 $\omega 9$	Elevated in EFA deficiency
Linoleic, $\omega 6$	(9,12-)18:2 $\omega 6$ (8,11,14-)20:3 $\omega 6$ (5,8,11,14-)20:4 $\omega 6$	Full EFA activity
Linolenic, $\omega 3$	(9,12,15-)18:3 $\omega 3$ (5,8,11,14,17-)20:5 $\omega 3$ (4,7,10,13,16,19-)22:6 $\omega 3$	Partial EFA activity

In this review the nomenclature most convenient to each discussion is used. The omega nomenclature is especially useful in describing interfamily competitive reactions. For acids of major nutritional significance an $\omega 6$ structure is required for full EFA activity. An objection to this nomenclature is that it implies that the terminal structure of a fatty acid determines its EFA activity. Prior to 1964 this implication was frequently stated in the literature. The discovery of EFA activity in odd-chain fatty acids has shown that the structural requirements for EFA activity are more complex.

III. Structural Requirements

A. DETERMINATION OF ESSENTIAL FATTY ACIDS ACTIVITY

The specific structural requirements for EFA cannot be determined until the precise metabolic role of the polyenoic acids is defined. Acids that relieve the symptoms produced in animals reared on a fat-free diet are said to have EFA activity. The two most common bioassays for EFA activity are relief of depressed weight gain and relief of dermal lesions. Weight gain is often monitored as "food efficiency," which is expressed as weight gained in grams per 100 gm of diet. Values for rats fed a fat-free diet compared to rats fed a diet containing linoleate are 23.7 ± 0.6 and 27.8 ± 0.6 gm gained per 100 gm diet, respectively. Dermal lesions are recorded as "dermal scores." Dermal scores for rats fed fat-free and linoleate diets are 2.8 ± 1.3 and 0.2 ± 0.2 , respectively (Rahm and Holman, 1964b).

The tissue and organ content of trienoic acids increase while tetraenoic acids decrease in animals fed EFA-deficient diets. Holman suggested that determination of the triene:tetraene ratio could be used as criterion for EFA deficiency. A triene:tetraene ratio less than 0.4 indi-

cated the animal was receiving at least the minimal EFA requirement (Holman, 1960). Tinsley (1964) showed, however, that the triene:tetraene ratio could be increased 4-fold in heart phospholipids and in liver mitochondria by increasing the level of dietary oleic acid to 15% of the calories with the animal showing no gross EFA deficiency symptoms.

The two chemical assays for EFA are hydroperoxide formation with soybean lipoxidase (Zmachinski *et al.*, 1966) and prostaglandin formation. Determination of prostaglandin from polyenoic acids is not strictly a chemical assay for EFA because the prostaglandins formed must be bioassayed for activity (Beerthuis *et al.*, 1968). These reactions are discussed in Section IV, J.

Recently, Ito and Johnson (1968) demonstrated that liver mitochondria from EFA-deficient rats oxidize NADH in hypotonic solution more slowly than mitochondria from normal rats. This reaction provides a simple biochemical criterion for EFA deficiency. However, the test does not distinguish between animals fed linoleic acid which has full EFA activity and linolenic acid which in rats has only partial EFA activity (Guarnieri and Johnson, unpublished results.) Decker and Mertz (1967) have studied some mitochondrial permeability properties that may also provide a simple biochemical criterion for EFA deficiency. When weanling rats were fed diets containing elaidic acid, t9-18:1 (t = trans), for more than 6 weeks, liver mitochondria swelled at a rate 2 to 3 times greater than mitochondria from rats fed control diets when the experiments were performed in hypotonic medium containing inorganic phosphate. It was not clear that the elaidic acid diets produced an EFA deficiency in the rat because no gross EFA deficiency symptoms were apparent. The efficacy of using mitochondrial swelling rates in hypotonic solution containing inorganic phosphate as a criterion of EFA deficiency awaits further study. Reactions involving membrane swelling and permeability properties are discussed in Section V.

It cannot be said that any bioassay for EFA deficiency is specific for EFA deficiency until the basic metabolic lesion(s) is (are) elucidated. Determination of gross changes are not always meaningful in nutritional studies that involve dietary interventions other than fatty acids. For example, EFA-deficient chickens compared to normal chickens have smaller body weight and repressed egg weight and production with zero hatchability. Menge (1967) demonstrated that the test diet lacked a factor(s) other than linoleate that was necessary for optimal reproduction. Dermal lesions must be assayed under tightly controlled temperature and humidity conditions (Aaes Jorgensen,

1961). Mitochondrial bioassay requires animal sacrifice, which becomes impossible for larger animals. Erythrocyte bioassays do not appear to be sufficiently critical (Section V).

B. POSSIBLE ENDOGENOUS PRECURSORS

Previous to the work of Beerthius *et al.* (1968), and Schlenk and co-workers (Schlenk *et al.*, 1964; Schlenk and Sand, 1967) only polyenoic acids of the $\omega 6$ and the $\omega 3$ family were known to have EFA activity. Many workers attempted to find possible endogenous precursors to linoleic or linolenic acid. These studies are summarized in Table I.

Murty *et al.* (1962) and Reiser *et al.* (1962) have reported that 2-8:1 $\omega 6$ is converted to 9,12-18:2 $\omega 6$ in the laying hen. Brenner *et al.* (1962) were not able to confirm, in rats, the conversion of 2-8:1 ($\omega 6$) to 9,12-18:2 ($\omega 6$). Anderson and Reiser (1966) repeated the study of Murty *et al.* (1962) and Reiser *et al.* (1962) with rats under the same conditions used with laying hens and could find no evidence in the liver for the conversion of 2-8:1 to 9,12-18:2. The octenoate, unlike octanoate which stimulates fatty acid synthesis, inhibited fatty acid synthesis. The only reported monoenoic precursor to EFA is, therefore, 2-8:1 ($\omega 6$) in the laying hen. The importance of this phenomenon is obscure.

Small but measurable amounts of 5,8-14:2 ($\omega 6$) are converted to linoleic acid in fat-deficient rats (Sprecher, 1968b). In HeLa cells 5,8-14:2 is readily converted to linoleic acid (Stoffel and Scheid, 1967). Rats readily convert 7,10-16:2 to longer $\omega 6$ polyenoic acids, and 7,10,13-16:3 and 4,7,10,13-16:4 to longer $\omega 3$ polyenoic acids (Sprecher, 1968b; Klenk, 1965a). Odd-numbered fatty acids of the $\omega 5$, $\omega 7$, and perhaps the $\omega 2$ series have EFA activity (Schlenk and Sand, 1967; Beerthius *et al.*, 1968). Since 14- and 16-carbon polyenoic acids are converted to longer-chain acids, perhaps 15-carbon polyenoic acids ($\omega 5$, $\omega 7$, and $\omega 2$) will also be extended.

C. TRANS FATTY ACIDS

Polyenoic acids with trans unsaturation do not have EFA activity, but trans acids occur in a variety of food sources (Kaufmann and Mankel, 1964) and are produced from cis acids by food-processing methods such as frying. A question of major nutritional significance has been: Do trans acids interfere with the metabolism of cis acids?

The trans isomer (t) of oleic acid, elaidic acid, or t9-18:1 is metabolized similar to oleic acid. These studies are summarized in Table IIa.

Table I
ESSENTIAL FATTY ACID PRECURSORS

Reference ^a	Precursor	Family	Enzyme source	Product
(1)	12:18:1	$\omega 6$	Rat <i>in vivo</i>	No 9,12-18:2
(2)	6:18:1	$\omega 12$	Rat <i>in vivo</i>	No 9,12-18:2
(3)	Acetate		Laying hen	No 9,12-18:2
(4)	2:8:1	$\omega 6$	Laying hen	9,12-18:2
(5)	2:8:1	$\omega 6$	Laying hen	9,12-18:2
(6)	2:8:1	$\omega 6$	EFA-deficient rat	No 9,12-18:2
(7)	2:8:1	$\omega 6$	EFA-deficient rat	No 9,12-18:2
(8)	4:10:1	$\omega 6$	EFA-deficient rat	No 9,12-18:2
(8)	3,6-12:2	$\omega 6$	EFA-deficient rat	No 9,12-18:2
(8)	5,8-14:2	$\omega 6$	EFA-deficient rat	9,12-18:2, minor amounts
(8)	7,10-16:2	$\omega 6$	EFA-deficient rat	9,12-18:2
(9)	7,10-16:2	$\omega 6$	Rat <i>in vivo</i>	9,12-18:2 and 5,8,11,14-20:4
(9)	7,10,13-16:3	$\omega 3$	Rat <i>in vivo</i>	5,8,11,14,17-20:5
(9)	4,7,10,13-16:4	$\omega 3$	Rat <i>in vivo</i>	5,8,11,14,17-20:5
(10)	3,6-12:2	$\omega 6$	HeLa cells	No 9,12-18:2
(10)	5,8-14:2	$\omega 6$	HeLa cells	9,12-18:2
(10)	7,10-16:2	$\omega 6$	HeLa cells	9,12-18:2
(10)	4,7,10-16:3	$\omega 6$	HeLa cells	9,12-18:2

^aKey to references:

1. Fulco and Mead (1960)
2. Sand *et al.* (1965)
3. Murty (1960)
4. Murty *et al.* (1962)
5. Reiser *et al.* (1962)
6. Bremner *et al.* (1962)
7. Anderson and Reiser (1966)
8. Sprecher (1968b)
9. Klenc (1965a)
10. Stoffel and Scheid (1967)

There are two reports on dissimilarities between oleic and elaidic acid metabolism. Willebrands and Van Der Veen (1966) reported that the perfused heart can completely oxidize oleic acid, but it only partially oxidizes t9-18:1 to t5-14:1. Munch (1965) reported that when rats were fed trieladin as the dietary lipid heart, liver and kidney phospholipids incorporate c5,t9-18:2 (c=cis).

Linoleic acid has 3, and linolenic acid has 7, trans isomers. There is some disagreement concerning the ability of these acids to be extended and desaturated. Overall these acids are metabolized similarly to the all cis isomers. Studies are summarized in Table IIb. Linoleic and linolenic acid isomers with one trans double bond (per-

Table IIa
THE METABOLISM OF THE OLEIC ACID TRANS ISOMER

Reference	Data
Mattson (1960)	No EFA activity
Dhopeshwarkar and Mead (1962), Bloomstrand <i>et al.</i> (1963)	Converted to stearic acid and oleic acid by cellular enzymes
Ono and Fredrickson (1964)	Intestinal metabolism similar to oleic acid
Lands (1965)	Acts like a saturated acid when acylating phospholipids
Alfin-Slater <i>et al.</i> (1965)	No effect on reproduction, life span, or cholesterol levels in rats

Table IIb
THE METABOLISM OF LINOLEIC AND LINOLENIC ACID TRANS ISOMERS

Reference	Data
Mattson (1960)	No EFA activity
Ono and Fredrickson (1964)	Intestinal metabolism similar to cis acid
Coots (1964)	—
Anderson and Coots (1967)	Completely oxidized to CO ₂ at rates similar to cis acid
Anderson (1968)	—
Blank and Privett (1963)	Extension and desaturation reactions
Brenner and Peluffo (1969)	—
Privett and Blank (1964)	—
Stearns <i>et al.</i> (1967)	—
Knipprath and Mead (1964)	—
Selinger and Holman (1965)	—
Privett <i>et al.</i> (1966)	Incorporated into phospholipids similar to saturated acids
Selinger and Holman (1965)	—
Lands <i>et al.</i> (1966)	—
Anderson (1968)	—

haps two) are converted to longer polyenoic acids. Therefore, these acids can compete with linoleic and linolenic acids for chain elongation and desaturation enzymes. It does not appear that trans acids suppress cis acid conversions to pathological levels if there is an adequate EFA supply. So, trans fatty acids do not have "anti" EFA activity.

The cholesterol esters of trans fatty acids are hydrolyzed to a lesser degree than cis unsaturated esters (Sgoutas, 1968). Although trans acids resemble saturated fatty acids, trans acids are not hypercholesterolemic (McMillan, 1964). Unlike cis acids, trans acids are not hypocholesterolemic (Tidwell *et al.*, 1965; Rand and Quackenbush, 1965). It is possible to conclude from these facts that the hypocholesterolemic effect of EFA is effected by a metabolic sequence that is available to cis but not to trans fatty acids, and that hypercholesterolemia is due to an absence of effects produced by unsaturation.

It should be pointed out here that there are at least three endogenous sources of trans double bonds in fatty acids. Polyenoic acids, linoleic acid for example, undergoes regular β -oxidation until the double bonds are in the 3,6 position of the thio ester. Δ -3-cis, Δ -2-trans-enoyl-CoA isomerase converts the 3-cis double bond to a 2-trans double bond, which is then hydroxylated and dehydrogenated, and a 2 carbon unit is removed by the 3-oxo-acyl-CoA-thiolase. The resulting Δ 4-cis thio ester is converted by an acyl-CoA-dehydrogenase to Δ -2-trans, Δ -4-cis thio ester, which again is hydroxylated, dehydrogenated, and cleaved (Stoffel *et al.*, 1964). Stoffel (1966) has shown that t2,c8,c11,c14-20:4 is an intermediate in the chain elongation of 6,9,12-18:3 to 8,11,14-20:3. The third source of trans unsaturation results from the conversion of polyenoic acids by a lipoxidase-type reaction to hydroxy acids containing a trans double bond. For example, incubation of 8,11,14-20:3 with microsomal and cytoplasmic enzymes and tetrahydrofolate afforded 15 hydroxy-c8,c11,t13-20:3 and 11 hydroxy-c8,t12,c14-20:3 (Hamberg and Samuelsson, 1967b). The details of this reaction are discussed in Section IV, J.

D. POSITIONAL ISOMERISM

Polyenoic acids having double bonds in various positions have been extensively studied to determine specific structural requirements for EFA. The criteria used to assay EFA activity of an isomer are relief of EFA deficiency lesions; chain extension and olefination; reaction with soybean liposidase and prostaglandin formation. Chain extension and olefination are not exclusive EFA properties, but all

acids with EFA activity are lengthened and desaturated [except long-chain members of a series, which are shortened and hydrogenated (Verdino *et al.*, 1964)].

EFA have no endogenous precursors. The double bonds, all cis, have a divinyl methane rhythm (cis, cis, 1,4-pentadiene arrangement). Acids are lengthened by the addition of two carbons at the carbonyl group. If it is assumed that all EFA are structurally related to linoleic acid, there should exist some structural pattern that relates to EFA activity. In the early 1960's several laboratories were performing the arduous task of synthesizing and testing various polyenoic acids for EFA activity. In Table III a series of acids are listed that were studied

Table III
LIPOXIDASE CATALYZED POLYENOIC ACID OXYGENATION

Acid	Family	Site of oxygen ^a introduction	Calcium activated ^b oxidation rate
9,12-18:2	ω6	C-13	100
9,12,15-23:3	ω8	No reaction	ND ^c
8,11,14-22:3	ω8	No reaction	ND
10,13,16-22:3	ω6	C-17	ND
4,7,10,13,16,19-22:6	ω3	C-17	93.6
9,12,15-21:3	ω6	C-16	ND
6,9,12,15-21:4	ω6	ND	44.6
5,8,11,14,17-20:5	ω3	C-15	91.4
5,8,11,14-20:4	ω6	C-15	76.8
2Me-5,8,11,14-20:4	ω6	ND	No reaction
15Me-8,11,14-20:3	ω6	No reaction	ND
8,11,14-20:3	ω6	C-15	ND
5,8,11-20:3	ω9	No reaction	ND
11,14,17-20:3	ω3	ND	55.7
11,14-20:2	ω6	ND	86.9
8,14-20:2	—	No reaction	ND
10,13,16-19:3	ω3	ND	83.3
10,13-19:2	ω6	ND	3.5
6,9,12,15-18:4	ω3	ND	27.6
5,8,11-18:3	ω7	ND	39.1
6,9,12-18:3	ω6	C-13	39.3
9,12,15-18:3	ω3	C-13	79.0
9,15-18:2	—	ND	32.6
5,8,11-17:3	ω6	ND	25.6
9,12-17:2	ω5	ND	19.3
6,9,12-16:3	ω4	ND	3.5

^aHamberg and Samuelsson (1967a).

^bHolman *et al.* (1969).

^cND = Not Determined.

by using the soybean lipoxidase reaction. With the significant exception of 9,12-17:2 ($\omega 5$) the reaction was specific for $\omega 6$ and $\omega 3$ fatty acids. Some other acids tested for EFA activity are listed in Table IV.

The task of correlating structure with EFA activity was almost impossible until the discovery by Schlenk and co-workers that odd-chained $\omega 5$ and $\omega 7$ fatty acids had EFA activity. The discovery immediately permitted several empirical generalizations concerning polyenoic acid metabolism.

1. Chain Extension and Olefination

A 16-, 17-, or 18-carbon acid with a Δ^9 double bond is olefinated more rapidly than acids with a single double bond at Δ^{11} . Acids of 17 or 18 carbon atoms that do not have a Δ^9 or Δ^{11} double bond are not further olefinated. In the liver there is no major extension of 17- or 18-carbon acids that do not have at least two double bonds. The presence of two double bonds with Δ^9 unsaturation does not guarantee further olefination or lengthening.

2. EFA Activity of 17- or 18-Carbon Acids

A $\Delta^{9,12}$ double bond system is required for EFA activity. A Δ^{15} double bond decreases EFA activity. (Possibly c9,c12,t15-18:3 would have more EFA activity than linolenic acid.)

3. EFA Activity of 20-, 21-, and 22-Carbon Acids

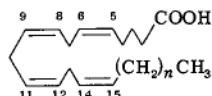
Acids of 20 and 21 carbon atoms must have an 11,14 double bond system for EFA activity. A 13,16 double bond system is required for 22-carbon acids.

Beerthius *et al.* (1968) discovered that odd-chain $\omega 5$ and $\omega 7$ fatty acids were converted to biologically active prostaglandins. With this information and from the accumulated positive and negative evidence on structure and EFA activity (Table IV), Beerthius and co-workers concluded the following: fatty acids with the structure shown in Fig. 1, and those which can be converted into it by the animal organism, are essential. This theory fits most present knowledge. Linolenic acid (and perhaps 9,12,15-17:3) has appreciable EFA activity. Linolenic acid is metabolized into a biologically active prostaglandin, E_3 . The hypothesis concerning the structure in Fig. 1 should be addended with the note that acids with $n=2$ or 1 have some EFA activity.

Exceptions exist. The odd-chain acid 9,12,15-21:3 reacts with soybean lipoxidase. Of the 13 possible isomeric octadecadienoates the 13,16 CoA isomer has a phospholipid acyltransfer rate almost two times greater compared to the rate of the EFA CoA isomer, 9,12 (Reitz

Table IV
EFA ACTIVITY OF POLYENOIC ACIDS

Acid	Family	Test for activity ^a						References ^b
		A	B	C	D	E	F	
15-16:1	ω_1	—	—					(1,3)
6,9,12,15-16:4	ω_1	—	—					(1)
10,13,16-18:3	ω_2	—	—	—	—	—		(2)
6,9,12,15-17:4	ω_2	+	+	+	+	+		(4)
9,15-18:2	ω_3	—	+	—	—	+		(5,8)
12,15-18:2	ω_3	—	+	—	—	—		(8)
9,12,15-18:3	ω_3	+	+	+	+	+		(5,7)
6,9,12,15-18:4	ω_3						+	(5)
10,13,16-19:3	ω_3						+	(5)
7,10,13,16-19:4	ω_3		+	+	+	+		(10)
11,14,17-20:3	ω_3	+	+	+	+	+	+	(5,7)
5,8,11,14,17-20:5	ω_3	+	+	+	+	+	+	(5,7)
6,9,12,15,18-21:5	ω_3			+	+	+		(9)
4,7,10,13,16,19-22:6	ω_3	+	+	+	+	+	+	(5,6,7,9)
9,12-16:2	ω_4	—	+	±	±	±		(4)
6,9,12-16:3	ω_4						+	(5)
8,11,14-18:3	ω_4	—					—	(2,10)
9,12-17:2	ω_5	+	+	+	+	+	+	(4,5)
6,9,12-17:3	ω_5	+	+	+	+	+		(4)
10,13-18:2	ω_5	—	—	—	—	—		(2,4)
8,11,14-19:3	ω_5	+	+	+	+	+		(4,10)
5,8,11,14-19:4	ω_5	+	+	+	+	+		(4,10)
6,9,12,15-20:4	ω_5			+	+			(9)
8,11-17:2	ω_6	—		—	—	—		(4)
5,8,11-17:3	ω_6						+	(5)
9,12-18:2	ω_6	+	+	+	+	+	+	(5,6,7)
6,9,12-18:3	ω_6	+	+	+	+	+	+	(5,6)
10,13-19:2	ω_6	—		±	±	+		(4,5,12,13)
7,10,13-19:3	ω_6	—		±				(11,12,13)
4,7,10,13-19:4	ω_6			±				(11)
11,14-20:2	ω_6	+	+	+	+	+	+	(5,7)
8,11,14-20:3	ω_6	+	+	+	+	+	+	(7)
5,8,11,14-20:4	ω_6	+	+	+	+	+	+	(7)
9,12,15-21:3	ω_6						+	(6)
6,9,12,15-21:4	ω_6				±	±		(9)
10,13,16-22:3	ω_6						+	(6)
4,7,10,13,16-22:5	ω_6	+	+	+	+	+	+	(7)
8,11-18:2	ω_7	—						(2)
5,8,11-18:3	ω_7	—		±	±	+		(2,5)
7,13-20:2	ω_7		+	—	—	—		(8)
4,7,10,13-20:4	ω_7	—		±				(10)
8,11,14-21:3	ω_7	+	+	+	+	+		(10)
5,8,11,14-21:4	ω_7	+	+	+	+	+		(10)
8,11,14-22:3	ω_8						—	(6)
9,12,15-23:3	ω_8	—	+	—	+	+	—	(6)
5,8,11-20:3	ω_9	—	+	—	+	+	—	(6,9,14)



$$n = 3, 4, \text{ or } 5$$

FIG. 1. Proposed structural model for polyenoic acids with essential fatty acid activity. From Beerthuis *et al.* (1968).

et al., 1968). Apparently acyltransferase specificity has features unrelated to present knowledge concerning polyenoic acid metabolism.

It is difficult to assess the nutritional significance of odd-chain fatty acids. Common food sources do not have large amounts of odd-chain fatty acids. However, Casal and Holman (1965) found that high dietary starch levels stimulated the appearance of a high proportion of several odd-chain fatty acids including 15:0, 15:1, 17:0, 17:1, 17:2, 19:0, 19:1, 19:2, 19:3, and 19:4. If odd-chain fatty acids have no nutritional significance, the only acids that have EFA activity are linoleic and linolenic family acids. EFA activity relates to the 9,12 double bond system in 18-carbon acids and the 11,14 double bond system in 20-carbon acids.

E. ESSENTIAL FATTY ACID DERIVATIVES

Highly unsaturated fatty acids are used to a great extent in food processing and preparation. Much work has been carried out on the nutritional effects of polyenoic acids used in food preparations. For example, there have been many studies on the effect of ingestion of oils used in frying food. Because these studies involved oils of relatively unspecified composition, they are not considered here.

The hexahydroxy derivatives of 9,12,15-18:3 have no EFA activity. Linoleyl and linolenyl alcohols stimulate growth, but only linoleyl

Footnotes to Table IV:

^aActivity test: A=relief of deficiency lesions; B=incorporation into phospholipid; C=prostaglandin formation (\pm =inactive prostaglandin); D=chain extension; E=desaturation; F=lipoxidase reaction.

^bKey to references:

- | | |
|-----------------------------------|------------------------------------|
| 1. Klenk (1965b) | 8. Sprecher <i>et al.</i> (1967) |
| 2. Klenk (1965a) | 9. Schlenk <i>et al.</i> (1967) |
| 3. Knipprath and Mead (1966) | 10. Beerthuis <i>et al.</i> (1968) |
| 4. Schlenk and Sand (1967) | 11. Van Dorp <i>et al.</i> (1964b) |
| 5. Holman <i>et al.</i> (1969) | 12. Rahm and Holman (1964b) |
| 6. Hamberg and Samuelsson (1967a) | 13. Thomasson (1962) |
| 7. Many references | 14. Walker (1966) |

alcohol cures dermal symptoms (cf. Aaes Jorgensen, 1961). Polyunsaturated alcohols are not converted to prostaglandins; a free carboxyl is essential for prostaglandin formation from polyenoic acids (von Euler and Eliasson, 1967 p. 33). Gundstone (1966) speculated that epoxy acids may be intermediates in polyenoic acid biosynthesis. Rahm and Holman found that 12:13 epoxy, 9-18:1 had no EFA activity (Rahm and Holman, 1964b). Elson *et al.* (1966) found that the cholesterol-lowering effect of vegetable oils may be seriously decreased if polyenoic acids are not 2-positioned on the glyceride. The cyclopropane derivatives of oleate, linoleate, linolenate, and arachidonate are not incorporated into the mitochondrial or microsomal phospholipids of EFA-deficient rats (Guarnieri and Johnson, unpublished results).

IV. Metabolism and Function of Polyenoic Acids

Inconsistencies in relating polyenoic acid structure to function as measured by the various EFA assays are related to the fact that polyenoic acids undoubtedly have a variety of functions. It is reasonable to assume that not all polyenoic (EFA) acid functions are known.

The known polyenoic acid functions can be considered in several arbitrary categories. Polyenoic acids act as an energy source similar to saturated and monoenoic acids. Stoffel and Schiefer (1965) found that saturated, monoenoic, and polyenoic acids are degraded by rat liver mitochondria at similar rates. Nutritionally, polyenoic acids have a hypolipemic action. This action takes place when the polyenoic acids supply about 5% of the dietary calories. EFA requirements are satisfied by milligram amounts of polyenoic acids. Polyenoic acid-induced hypolipemia seems to be a mass effect of dietary unsaturation. Until there is further insight into polyenoic acid function at the molecular level, any attempt to correlate EFA and hypolipemia would be hazardous.

A. POLYENOIC ACID INTERACTIONS

Polyenoic acids function as structural components in lipoprotein membranes. Also, they are converted into prostaglandins. These functions are discussed in Sections V and IV, H, respectively. The polyenoic acid function most difficult to describe (in terms of the EFA deficiency syndrome) is the chain lengthening and desaturation reactions. Largely through the excellent studies of Holman and co-workers it is now understood that extension and olefination reactions are dynamic, not static, polyenoic acid functions. That is, polyenoic

acid conversions regulate the metabolism of other acids through competition for the enzymes involved in the conversions. The tissue polyenoic acid composition is, therefore, dependent upon the dietary supply of polyenoic acids and is regulated by competitive inhibition of their metabolism. For example, in the absence of EFA, oleic acid is extended and desaturated. Linoleic acid and its metabolites rapidly replace oleic acid and its metabolites when the EFA-deficient animal is fed linoleic acid. A graph clearly illustrating this competitive inhibition in polyenoic acid metabolism is shown in Fig. 2. As the dietary linoleate level increases from 0 to 5% of the dietary calories, the heart tissue level of 9,12-18:2 and 5,8,11,14-20:4 ($\omega 6$) increase from about 2 to 15% of the total fatty acids. Simultaneously, 9-18:1 and 5,8,11-20:3 ($\omega 9$) content decreases from 30 to 20% and 14 to 1%, respectively.

Competitive inhibition in polyenoic acid metabolism has been extensively verified. A brief summary of these studies is contained in Table V. Although intrafamily polyenoic acid metabolism inhibition has been demonstrated, the majority of the studies concern interfamily polyenoic acid metabolism inhibition. The omega nomenclature (i.e., oleic acid and linoleic and linolenic acid metabolites are $\omega 9$, $\omega 6$, and $\omega 3$ acids, respectively) is very convenient in depicting interfamily and intrafamily competitive inhibitions and metabolisms. This nomenclature will be used in the following discussion.

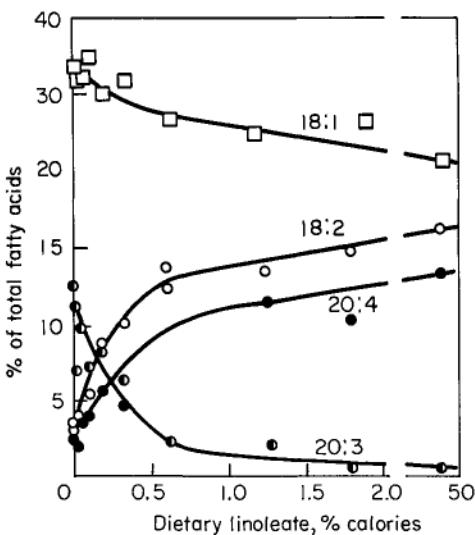


FIG. 2. Competitive inhibition in the metabolism of polyenoic acids. From Holman (1964).

Table V
COMPETITIVE INHIBITION IN POLYENOIC ACID METABOLISM

Reference	Inhibited conversion to higher polyenoic acid	Inhibitor
Holman (1964)	18:1 ω 9 18:1 ω 9 18:1 ω 9 18:2 ω 6 18:2 ω 6 18:3 ω 3 20:4 ω 6 16:0; 18:0	18:2 ω 6 20:4 ω 6 18:3 ω 3 18:1 ω 9 18:3 ω 3 18:2 ω 6 18:3 ω 3 16:0; 18:0
Brenner and Peluffo (1966b)	18:1 ω 9 18:1 ω 9 18:2 ω 6 18:2 ω 6 18:3 ω 3 18:3 ω 3	18:2 ω 6 18:3 ω 3 18:1 ω 9 18:3 ω 3 18:1 ω 9 18:2 ω 6
Lowery and Tinsley (1966a)	20:3 ω 3	20:3 ω 9
Lowery and Tinsley (1966b)	18:1 ω 9 18:2 ω 6	18:2 ω 6 18:1 ω 9
Brenner and Peluffo (1966a)	18:2 ω 6 18:3 ω 3	22:6 ω 3 22:6 ω 3
Mohrhauer <i>et al.</i> (1967b)	18:2 ω 6	Saturated acids
Mohrhauer and Holman (1967)	18:2 ω 6	Monoenoic acids
Uchiyama <i>et al.</i> (1967)	18:0 18:0 18:0	18:1 ω 9 18:2 ω 6 18:3 ω 3

Studies on polyenoic acid interactions have been considerably refined. Caster *et al.* (1966) prepared 21 dietary fatty acid ester mixtures from common fats and oils plus small amounts of purified fatty acid esters. Correlations between diet and tissue fatty acids were computed with the aid of a digital computer. Equations were developed for estimating the amounts of 4 dietary fatty acids from tissue analysis and of 7 tissue fatty acids from diet analysis. The data suggested that there are 4 independent dietary fatty acid variables: short-chain saturated (6:0-14:0), long-chain saturated (16:0-22:0), linoleic and linolenic acids. Lindstrom and Tinsley (1966) have developed a mathe-

matical model system for studying the interactions in polyenoic acid metabolism. The model assumes that simple competitive inhibition occurs between parent acids as they are lengthened and dehydrogenated to their derivative acids. A difficulty in creating such a model system is that assumptions must be made concerning the enzymes involved in polyenoic acid metabolism. These enzymes or enzyme complexes have not been isolated; therefore, assumptions concerning their kinetics must be viewed with caution.

Polyenoic acid conversion to higher metabolites involves chain lengthening and desaturating steps. The endoplasmic reticulum is the major site of polyenoic acid chain lengthening and desaturation enzymes (Stoffel and Ach, 1964; Mohrhauer *et al.*, 1967a). Both lengthening and desaturating steps respond to dietary inhibition, but in different ways. The steady-state concentration of acids formed by dehydrogenation steps respond to dietary inhibition in a sharply exponential manner, whereas those formed by chain lengthening respond weakly, in a seemingly linear fashion (Holman, 1964). Brenner and Peluffo (1966b) studied the oxidative desaturation of labeled acids using rat liver microsomes. Only unsaturated acids of similar chain length significantly depressed the conversion of oleic, linoleic, and linolenic acids into their corresponding higher polyenoic acids. Approximate K_m and K_i values for the conversion of acids in the presence and absence of other acids indicated the competitive phenomena could be located at the desaturating step. Mohrhauer *et al.* (1967a), Marcel *et al.* (1968), and Christiansen *et al.* (1968) have studied the chain elongation of 18:2 ω 6, 18:3 ω 3, and 18:3 ω 6 in the presence of other fatty acids. Under anaerobic conditions chain elongation could be quantitatively separated from dehydrogenation, which otherwise required the same reaction system. Of the 20 different acids tested only 21:0 and 22:0 had no inhibitory effect on 18:2 ω 6 elongation: 14:0 and 15:0 were the most effective inhibitors. Since only 18:1 ω 9, 18:3 ω 3, and 22:6 ω 3 inhibit 18:2 ω 6 conversion to 18:3 ω 6, it appears that dehydrogenation enzymes are much more substrate-specific than chain elongation enzymes. However, since 18:3 ω 6 is elongated much faster than 18:3 ω 3, it is apparent that double bond position rather than double bond number is the important factor governing polyenoic acid reaction with chain-lengthening enzymes. Different acids inhibited the elongation if 18:3 ω 3 and 18:3 ω 6 in different fashions (Christiansen *et al.*, 1968). Studies using whole microsomes as the enzyme source are greatly complicated by the fact that two enzyme systems, the chain lengthening and desaturating and the phospholipid acylating system are competing for the fatty acid substrate (Brenner and

Peluffo, 1969; Brenner *et al.*, 1969; Nakagawa and Uchiyama, 1969; Nervi *et al.*, 1968). Ethylenic bond position effects not only the desaturation reaction, but also the phospholipid acylation reaction (Reitz *et al.*, 1968).

B. BIOSYNTHESIS

The microsomal enzymes that catalyze chain extension and olefination reactions have not been isolated. There are a number of different mechanisms for fatty acid desaturation in a variety of organisms (Bloch, 1969). It seems likely that enzymes desaturating saturated acids are different from enzymes that desaturate polyenoic acids. The major difficulty in isolating the lengthening and desaturation enzymes is that the enzymes are bound to microsomal membranes, and an intact membrane structure appears to be necessary for their activity. Also, the desaturating enzymes seem to require some connection to microsomal electron transfer components.

The most effective system for polyenoic acid lengthening requires malonyl coenzyme A, NADPH, and ATP. Oxygen is required for desaturation (Mohrhauer *et al.*, 1967a). NADH can replace NADPH in chain-lengthening reactions (Nugteren, 1965). NADH and ascorbate can act as electron donors in stearyl-CoA desaturation (Oshino *et al.*, 1966). The requirements for oxygen and a reducing equivalent in the desaturation reaction imply an enzyme system similar to microsomal drug hydroxylation enzymes. However, the desaturation reaction does not involve the hemoprotein, P-450. An unknown cyanide-sensitive factor appears to be involved in the desaturation mechanism. The cyanide-sensitive factor may have an oxygen activating role via an iron-oxygen complex (Oshino *et al.*, 1966; Stoffel and Schiefer, 1966). The mechanism for the desaturation of unsaturated acids is unknown. Direct hydrogen removal at the site of the new double bond is the simplest mechanism. These hydrogens, compared to substrates for other dehydrogenases are relatively inert. Intermediates such as [II] in Fig. 3 may take part in the reaction. An extremely interesting phenomenon in fatty acid desaturation reactions is that the desaturation is depressed in the liver of alloxan-diabetic rats. Gellhorn and Benjamin (1964) first demonstrated this effect in the desaturation of 18:0 to 18:1 ω 9. Mercuri *et al.* (1967) demonstrated the effect in the desaturation of 18:2 ω 6 to 18:3 ω 6, 18:3 ω 3 to 18:4 ω 3, 18:1 ω 9 to 18:2 ω 9, and 16:0 to 16:1 ω 7. When the diabetic rats were injected with insulin the desaturating activity was recovered, but insulin had no effect *in vitro*. Retinal fatty acid synthesis is similarly affected by alloxan diabetes (Futterman *et al.*, 1968).

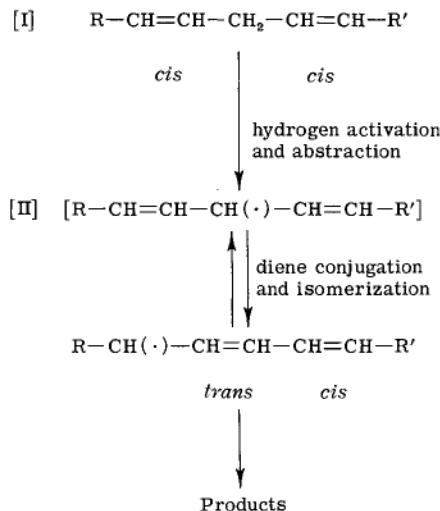


FIG. 3. Initial mechanism of lipid peroxidation. From Barber and Bernheim (1967).

Retroconversion of polyenoic acids by partial degradation and hydrogenation does not necessarily indicate that specific chain lengthening and desaturating enzymes are reversible. Retroconversion of 22:5 ω 6 to 20:4 ω 6 was first demonstrated by Verdino *et al.* (1964). Retroconversion has also been demonstrated with 22:4 ω 6; 22:4 ω 7; 11, 14-20:2 and t11, t14-20:2 (Sprecher, 1967, 1968a; Stearns *et al.*, 1967). Biohydrogenation occurs at the double bond closest to the carboxyl group (Schlenk *et al.*, 1967).

C. PHOSPHOLIPID METABOLISM

Phospholipids are the major repository of polyenoic acids, and the metabolic interrelations between the two are complex. Hundreds of enzymes may be involved.

When EFA-deficient rats were fed EFA, both linoleate and arachidonate displaced oleate in the 1-phospholipid position, but only arachidonate quantitatively replaces 2-positioned 20:3 ω 9 (Brenner and Nervi, 1965; Brenner and Jose, 1965). The decline in phospholipid ω 9 fatty acids by the dietary intervention of ω 6 fatty acids apparently results from the preferred esterification of ω 6 acids to the phospholipid 2-position (Johnson *et al.*, 1967). The dietary linoleate content directly influences the composition of the 2-position fatty acid and indirectly influences 1-positioned fatty acids (Moore *et al.*, 1965). In a

more detailed analysis Pudelkewicz and Holman (1968) showed that the dietary polyenoic acid influence on phospholipid 2-positioned fatty acids is different for different polyenoic acid families.

Lands and co-workers have shown that the acyl transfer rates from acyl-CoA esters to lysophosphatides are highly specific for the structure of the fatty acids. Saturated fatty acids are esterified in the 1-position; polyenoic acids are esterified in the 2-position. Detailed studies using the 16 isomeric octadecenoic acids and the 13 isomeric octadecadienoic acids have shown that the acyl-transferases acting at both the 1- and the 2-positions of lysolecithin discriminate between the isomers in very different ways (Reitz *et al.*, 1968, 1969). Similar results were reported by Van Deenen and co-workers (Van Den Bosch *et al.*, 1968b, 1969).

Phospholipases obviously play a key role in phospholipid-fatty acid metabolism. The study of these enzymes is one of the most active areas in phospholipid research. Depending on the tissue source the enzyme(s) has a variety of properties and activities. The phospholipase literature has not been reviewed comprehensively. The discussion given below is intended to alert the reader to some current areas of phospholipase research. Only phospholipases that hydrolyze fatty acids, phospholipase A, are covered.

Van Deenen and co-workers have extensively studied phospholipase chemistry. The enzyme from porcine pancreas has a zymogen form with a molecular weight of 15,000. The active enzyme has a molecular weight of about 13,800. Both forms have a high content in α -helix (Scanu *et al.*, 1969). The rat liver enzyme has activities that result in the deacylation of both 1- and 2-positioned fatty acids (Van Den Bosch *et al.*, 1968a). Bovine adrenal medulla lysosomes contain two phospholipases with pH optima at 4.2 and 6.5. Both enzymes were inhibited by about 10 mM calcium. They had different activities with phosphatidylcholine compared to ethanolamine (Smith and Winkler, 1968). Phospholipase isolated from *Naja naja* venom exists in 6 forms with molecular weights of 8500-22,000 (Salach *et al.*, 1968). Phospholipase from *Crotalus adamanteus* has two forms, both with a molecular weight of 30,000 (Wells and Hanahan, 1969).

Rat liver has two lysosomal phospholipases in addition to the mitochondrial phospholipase (Waite *et al.*, 1969). The mitochondrial phospholipase is located in the outer membrane fraction (Nachbauer and Vignais, 1968). Waite and Van Golde (1968) have reported that EFA-deficient mitochondria exhibit a higher phospholipase activity compared to normals.

Since phospholipase activity is stimulated by several agents that

stimulate mitochondrial swelling, the suggestion has been made that the two activities are related (Waite *et al.*, 1969). Since no enzyme has been associated with mitochondrial volume changes, the possibilities of this suggestion are exciting. However, there are a multitude of factors that affect mitochondria swelling, and there are several types of swelling. Fatty acids, the products of the phospholipase reaction, are mitochondria substrates and lytic agents under certain conditions. Suggested mechanisms relating mitochondrial swelling to phospholipase activity should be viewed with great care.

At present there is some disagreement concerning at what point in phospholipid synthesis the fatty acids acquire their nonrandom distribution, i.e., saturated fatty acids in the 1-position; polyenoic acids in the 2-position. Lands and Hart (1965) and Hill *et al.* (1968) reported that the fatty acids in phosphatidic acid had a random distribution. Cleland and co-workers concluded that the preferential distribution of fatty acids in phospholipids did not result from any specificity of the microsomal enzyme acylating glycerol 3-phosphate (Zahler and Cleland, 1969). These observations differ from the results of Possmayer *et al.* (1969), who found that phosphatidic acid isolated from rat liver had an asymmetrical fatty acid distribution. Most likely these contradictory observations will be explained by answers to the problem of phospholipid subspecies.

Collins (1960, 1963) was the first to observe that a phospholipid could be divided into subspecies dependent on its fatty acid content. Subsequent studies showed (Collins, 1962) that individual phospholipid metabolism was affected by the fatty acids present in the phospholipid, i.e., the phospholipid molecular species. The phosphatidylcholine species containing dienoic acids most actively incorporates phosphate, glycerol, and choline. The hexaenoic phosphatidylethanolamine species incorporates glycerol and phosphate most rapidly. In both phospholipids, tetraenoic subfractions have the lowest reactivity (Kanoh, 1969). Hexaenoic phosphatidylcholine is the major product of rat liver phosphatidylethanolamine methylation (Arvidson, 1968; Kanoh, 1969; Tinoco *et al.*, 1968; Lyman *et al.*, 1969b). The reincorporation of choline methyl groups into lecithin (Spitzer *et al.*, 1969), but not choline phosphotransferase (Mudd *et al.*, 1969), is affected by phospholipid molecular species.

At present it is not possible to distinguish which, if any, metabolic pathway predominates for phospholipid fatty acid incorporation. The type of phospholipid molecular species may affect the various pathways differently (Van Golde *et al.*, 1968). This type of control mechanism may furnish a partial explanation for the existence of molecular

species. A second explanation (Section IV, J) is that certain species may peroxidize and react with proteins in lipoprotein membranes to regulate membrane metabolism. Regardless of the explanation for the existence of individual molecular species, it is becoming increasingly apparent that phospholipid-fatty acid interactions are complex. Computer programs must be designed to analyze the interactions.

Phospholipid-fatty acid interactions obviously depend on the nature of the fatty acid pool, which in turn depends on lipid nutrition. However, the ratio of individual phospholipids is not a dietary variable. Great differences exist in the individual molecular species of phospholipids between normal and EFA-deficient rats (Van Golde *et al.*, 1968; Lyman *et al.*, 1969b), but the rate and extent of methyl incorporation into the total phosphatidylcholine is similar for both groups (Lyman *et al.*, 1969b).

There is competitive inhibition in polyenoic acid metabolism, and there is an effect of preferred acylation in phospholipid synthesis. Animals can be fed diets containing high ratios of oleic or linolenic acid to linoleic acid without showing gross EFA deficiency symptoms. Since linolenic acid is a strong inhibitor of linoleate metabolism (Holman, 1964), it might be expected that the preferred acylation of $\omega 6$ fatty acids to the 2-position of phospholipids is the primary mechanism in regulating tissue fatty acid content. It is difficult to relate preferred phospholipid acylation of $\omega 6$ acids to the EFA deficiency syndrome. A number of enzymatic reactions are known to have a requirement for phospholipids containing unsaturated acids. However, the unsaturated fatty acid requirement is not specific for EFA (De Pury and Collins, 1966).

Phospholipid acylating enzymes have a greater affinity for linoleic acid than for linolenic or oleic acid. This affinity is dependent on the linoleic acid structure. Guarnieri and Johnson made an attempt to separate the structural from the metabolic functions of polyenoic acids. Methylene bridges were added stereospecifically across the double bonds of several unsaturated acids using the Simmons-Smith reaction (LeGroff, 1964). The cyclopropane acids thus formed retain many structural and electronic characteristics of the original unsaturated acid. Since the ring configuration is cis and has appreciable sp^2 character (Bennett, 1967), it appeared that cyclopropane acids would be ideal compounds for studying the various functions of unsaturated acids. The cyclopropane derivatives of oleic, linoleic, linolenic, and arachidonic acid as methyl esters were fed to EFA-deficient rats (100-200 mg/day) for 1 week.

Fecal analysis showed that less than 10% of any cyclopropane acid was excreted. Cyclopropane fatty acids were identified in combined adipose tissue extracts by gas chromatography—carbon numbers for the appropriate cyclopropane fatty acid agreeing with those published by Christie and Holman (1966), and by infrared analysis—cyclopropane peaks at 1.65 and 2.23 μ (Gassman, 1962). However, we could not detect the presence of the fed cyclopropane acids and/or their metabolites in the mitochondrial or microsomal lipids (Guarnieri and Johnson, unpublished results).

The fact that cyclopropane acids are incorporated into adipose tissue lipids, which are mainly triglycerides—not into mitochondria and microsomes, which are mainly phospholipids—raises questions concerning the nature of the enzymes responsible for incorporating fatty acids into triglycerides and phospholipids. It is possible that feeding more cyclopropane acids for greater time periods would afford cyclopropane acid incorporation into phospholipids. However, Chung (1966) incubated 9,10-methylene- $^{14}\text{C}-\text{cis}$ -9,10-methylenehexadecanoic acid with mitochondria and was able to account for only 73 out of 1810 counts per minute as the metabolic by-product. Phospholipids will not incorporate cyclopropane fatty acids, but it may still be possible to obtain polyenoic acid structure-function information separate from metabolic-function information by feeding synthetic, cyclopropane fatty acid containing phospholipids.

D. COMPARATIVE METABOLISM OF LINOLEATE AND ARACHIDONATE

There are indications that arachidonic acid is the only essential fatty acid because feeding arachidonate to EFA-deficient rats completely cures all deficiency symptoms while the linoleic acid tissue content remains at a level found in EFA-deficient animals (Rahm and Holman, 1964b). These results should be interpreted with consideration to the more recent finding that polyenoic acids can undergo retroconversion. Prostaglandin E₁ is formed from 8,11,14-20:3 (ω 6) which is an intermediate in the conversion of linoleic acid to arachidonic acid. Arachidonic acid has a slower turnover time than linoleic acid (Coats, 1965; Brenner and Nervi, 1965). Phospholipids are metabolized less rapidly than triglycerides (Olivercrona, 1962), and triglyceride synthesis is increased in EFA-deficient rats (Solyom *et al.*, 1967). The greater retention of arachidonate compared to linoleate correlates with the conclusion of Catala and Brenner (1967) that triglycerides contain the

largest linoleic acid pool while the arachidonic acid pool is largest in phospholipids. Phospholipids containing arachidonate have the slowest turnover time (Collins, 1962), but cholesteryl arachidonate has the fastest turnover rate (Swell and Law, 1965). Arachidonic acid is several times more effective than linoleic acid in curing EFA deficiency lesions (Mohrhauer and Holman, 1963a). The proportion of liver arachidonate decreases in EFA deficiency because the weight of total phospholipid fatty acids increases as liver weight increases; however, the milligrams of arachidonate remain essentially constant (Ostwald *et al.*, 1965). There is an inverse relationship between relative amounts of linoleic acid and the total amount of 20-22-carbon $\omega 6$ polyenoic acids in red blood cell total phospholipids (Phillips, 1968).

E. ESSENTIAL FATTY ACIDS AND VITAMINS

The cofactor requirements for the known polyenoic acid reactions have only recently been studied. Chain extension and desaturation reactions require malonyl-CoA, reduced pyridine nucleotides, and ATP (Mohrhauer *et al.*, 1967a). Prostaglandin formation does not appear to require cofactors although conversions are enhanced in the presence of SH-containing compounds (von Euler and Eliasson, 1967, p. 33). These relatively simple cofactor requirements explain the negative results of earlier studies that attempted to find interrelations between various vitamins and EFA deficiency. Studies that attempt to analyze polyenoic acid metabolism in the presence of a vitamin deficiency are especially difficult to interpret. Vitamin deficiency often imposes a stress on the animal far greater than EFA deficiency.

Studies on the relation of polyenoic acids to vitamins that are not listed above as cofactors in polyenoic acid metabolism are not covered in this review. Pyridoxine and vitamin E studies are the most noticeable omissions. Jensen (1968) has reviewed the effects of vitamin E and EFA on avian reproduction. Horner and Morton (1961) found liver vitamin A stores of fat-deficient male rats fed on a diet containing 1% cholesterol not significantly different from those of controls which received (100 mg/day) linoleic acid. The liver vitamin A stores of fat-deficient female rats fed on a 1% cholesterol diet were 48% lower than those of their controls which had been fed linoleic acid. Hands *et al.* (1965) found that EFA-deficient rats had no vitamin A stores in their liver. Controls had approximately 190 IU/gm. Compared to normals, EFA-deficient rats had less visual acuity. Seward *et al.* (1966) found that tri-*o*-cresyl phosphate, a compound that decreases the liver content of vitamin A and vitamin E, accentuated EFA deficiency

symptoms and decreased the time of onset of dermal lesions. The biological half-life of liver ubiquinone is doubled in advanced EFA-deficient rats (Lee *et al.*, 1964). A choline deficiency does not appear to alter liver fatty acid patterns (Glende and Cornatzer, 1965). However, the conversion of linoleic acid to arachidonic acid is impaired in rats fed low levels of ethionine (Lyman *et al.*, 1968a, 1969a). The use of ethionine to inhibit polyenoic acid extension and olefination reactions may prove to be a very powerful tool in the study of polyenoic acid metabolism. Biotin-deficient, EFA-deficient chicks incorporate fatty acids into phospholipids at a greater rate than they incorporate fatty acids into triglycerides (Donaldson, 1966). Growth studies afford no evidence that a high level of dietary fat reduces the growth response to limiting intakes of pantothenate or increases the pantothenate requirement for maximal growth (Williams *et al.*, 1968).

F. ESSENTIAL FATTY ACIDS AND HORMONES

Many workers have noted hormone effects in EFA-metabolism. The nature and extent of the differences are not clear. Removal of endocrine glands probably stresses experimental animals more compared to an EFA-deficiency. Results from studies utilizing hormonal treatment are difficult to analyze because little is known concerning the target site(s) of most hormones.

Decreased prostaglandin production plus the general stress caused by an EFA deficiency may account for sterility of EFA-deficient animals. Evidence for such a hypothesis is lacking. Ahluwalia *et al.* (1967) found no qualitative or quantitative differences in seminal vesicle fatty acid composition between normal and EFA-deficient rabbits. It was suggested that there was a primary impairment of anterior hypophyseal function. There is a significant increase in androgen biosynthesis from cholesterol in EFA-deficient testes. This could be due to an increased mitochondrial permeability to cholesterol (Ahluwalia *et al.*, 1968). Testes appear to be more sensitive to EFA deficiency during development compared to maturation. These results suggest that secondary effects of EFA deficiency are responsible for eventual testes degeneration (Bieri *et al.*, 1969). Hypophysectomy caused a marked increase in testicular neutral lipid percentages and a slight increase in liver polar lipid percentages in EFA-deficient rats. There was little change in characteristic fatty acid interconversion patterns on feeding linoleate (Jensen *et al.*, 1968). Linoleate ($\omega 6$) and linoleinate ($\omega 3$) metabolism is greatly affected by thyroid treatment. Hyperthyroidism did not greatly affect hepatic and plasma $\omega 3$ fatty acid

levels. Hyperthyroid rat heart contained 425% more 4,7,10,13,16,19-22:6 (ω 3) than did that of its euthyroid control. Heart, plasma, and liver had increased arachidonate (ω 6) levels. Thyroid hormone appears to accelerate polyenoic acid extension and olefination (Peifer, 1968). Dietary calcium has a hypolipemic effect in rats fed saturated or unsaturated fat (Yacowitz *et al.*, 1967). Gamba and Quackenbush (1968) have suggested that the control mechanism that regulates the secretion of thyroidal iodine is dependent on essential fatty acids.

Many workers have noted a sex difference in EFA-deficient metabolism. The nature and extent of the difference is not clear. Morton and Horner (1961) found that deficient female rats had a greater liver cholesterol ester and triglyceride concentration than deficient males. Phospholipid arachidonate decreased equally in males and females. Phospholipid linoleate decrease and eicosatrienoate (ω 9) increase was greater in males. Liver triglyceride and cholesterol ester linoleate decrease and eicosatrienoate (ω 9) increase was greater in males, but arachidonate decrease was greater in females. Ostwald, Lyman, and co-workers found in rats fed diets high in saturated, or polyenoic acids characteristic sex differences both in the concentration of the different liver and plasma lipids and in their fatty acid pattern. Females grew more slowly than males and, therefore, exhibited EFA deficiency signs later. Females maintained higher proportions of arachidonate in phospholipids for longer periods than males. Females had lower amounts of liver cholesterol ester and much larger amounts of plasma cholesterol arachidonate than males. Ostwald *et al.* did not find that gonadectomy increased esterification of cholesterol with saturated acids. Males were more susceptible to EFA deficiency than females. In castrated animals estradiol caused a significant rise in plasma phospholipids and triglycerides compared to testosterone treatment. Ostwald and associates suggested that the observed sex differences could be more closely attributed to the smaller size of the female than to the presence of estradiol (Ostwald *et al.*, 1965; Okey *et al.*, 1966; Lyman *et al.*, 1966).

Aftergood and Alfin-Slater (1965, 1967) have suggested that estradiol may enhance esterification of cholesterol with more unsaturated acids and thus promote cholesterol transport and metabolism. Plasma cholesterol esterification was higher in the mature female rat than in the mature male. No sex differences were observed in animals fed fat-free diets. The suggestion was made that cholesterol may inhibit the formation of arachidonic acid from linoleic acid.

Ostwald and Lyman (1968) have shown that EFA-deficient rats exhibit the same kinds of sex differences as do rats on complete diets.

The differences appear to be influenced by estrogen. Female rats have higher amounts of the liver lecithin species that contains stearate plus arachidonate compared to males. Estradiol but not testosterone causes an increase in the incorporation of methionine into lecithin (Lyman *et al.*, 1968b). Male and female rats have different linoleate requirements. Female rats have about 1.3 to 1.6 times more polyenoic acid double bonds than males (Pudelkewicz *et al.*, 1968). There is a significant sex difference in the elongation of palmitic to stearic acid. Female rats performed the elongation with a capacity about twice as high as that of male rats. No sex differences were detected in the conversion of linoleic acid to arachidonic acid or in the incorporation of arachidonic acid into phospholipids. The enhanced female elongation of palmitic acid could not be traced to any direct estrogenic hormonal action (Christiansen *et al.*, 1969).

G. POLYENOIC ACIDS AND CHOLESTEROL ESTERS

Cholesterol addition to EFA-deficient diets causes deficiency symptoms to appear sooner compared to deficient diets without cholesterol (Takasugi and Imai, 1966). Cholesterol feeding aggravates the deficiency more in males than females. The formation of cholesterol ester by the plasma lecithin:cholesterol ester acyl transferase reaction has recently been reviewed by Glomset (1968). The reaction appears to be the physiologically important source of plasma cholesterol esters. Linoleic acid feeding compared to saturated fatty acids leads to an initial *increase* in cholesterol production, but the total plasma cholesterol is decreased due to an increased plasma cholesterol turnover. Plasma cholesterol linoleate level increases and cholesterol polyenoates have a faster turnover compared to saturated cholesterol esters (Boyd, 1962; Pinter *et al.*, 1964; Swell and Law, 1965). Plasma cholesterol ester levels are lowered following estrogen treatment, polyenoates being lowered to a greater degree than saturated cholesterol esters (Boyd, 1962). Plasma cholesterol esterification activity was found to increase, liver cholesterol esterification activity to decrease and plasma lipid phosphorus to decrease during an EFA deficiency (Sugano and Portman, 1965).

Sugano and Portman (1965) proposed that the differences in plasma and liver esterification activities induced by EFA deficiencies were secondary to alterations in hepatocytic organelle stability: altered stability of membranous components could result in an increased release rate of cholesterol ester synthesizing enzyme to the circulation. The lowered blood cholesterol levels in rats fed polyunsaturated fats

cannot be explained on the basis of an increasing accumulation in the liver, with a resultant inhibition of its formation. More cholesterol appears as a result of polyenoic acid ingestion, and it is not stored ultimately in the tissues (Pawar and Tidwell, 1967). Liver mitochondrial and microsomal enzymes in the presence of CoA and ATP esterify cholesterol with saturated and monoenoic acids. Liver cytosol enzyme(s) requiring no cofactor esterify cholesterol with polyenoic acids. In fasted rats cholesterol arachidonate synthesis by cytosol enzyme(s) increases 3-fold compared to fed rats (Swell and Law, 1967). Hyun *et al.* (1969) suggested that pancreatic cholesterol esterase is responsible for both synthesis and hydrolysis of the ester. The relative rates of ester formation were oleate, 100; linoleate 71; palmitate 56; stearate 43; and laurate 11. The rates for ester hydrolysis were oleate, 100; palmitate, 107; stearate, 104; linoleate, 55.

Obviously the interplay between polyenoic acids phospholipids, cholesterol, cholesterol esters, and hormones is exceedingly subtle and complex. Interpretation of this interplay is difficult, but the following patterns seem to emerge. Each phospholipid species has a characteristic fatty acid composition. Dietary lipids are a major determinant of the phospholipid fatty acid composition. The formation of lecithin from phosphatidylethanolamine is influenced by the phosphatidylethanolamine fatty acid composition. Estrogens (women are less susceptible to atherosclerosis than men) influence lecithin formation. Plasma lecithin:cholesterol acyl transferase reaction appears to be a major factor controlling cholesterol ester metabolism. Cholesterol ester formation is a determinant of plasma cholesterol metabolism.

H. PROSTAGLANDINS

An extensive literature has developed describing the chemistry and biochemistry and physiological activities of the prostaglandins, and the following reviews have appeared: Bergstrom *et al.* (1968); Ramwell *et al.* (1967); Bergstrom (1966, 1967a,b); Bergstrom and Samuelsson (1965); Horton (1969). A method for enzymatic analysis of prostaglandins has recently been reported (Anggard *et al.*, 1969).

The demonstration that prostaglandins can arise *in vivo* from the essential unsaturated fatty acids (Bergstrom *et al.*, 1964a; Van Dorp *et al.*, 1964b) has led to speculation concerning whether they might be the mode of expression of the latter. Attempts to demonstrate this have so far generally been without success.

Neither prostaglandins E₁, E₂, nor F_{2a} have been shown to influence the gross symptoms of an essential fatty acid deficiency in rats or mice.

Administration of the hormone by mouth, intravenous injection, and by intravenous infusion have been employed equally unsuccessfully (Gottenbros *et al.*, 1967; Kupiecki and Weeks, 1966). Similarly Du and Johnson (personal communication) failed to observe any sparing effect of prostaglandin E₁ injected intraperitoneally on either linoleic acid, a mixture of linoleic and linolenic acids, or corn oil, using rat liver mitochondrial respiratory control (Ito and Johnson, 1964) and oxidation of DPNH in hypotonic media (Ito and Johnson, 1968) as end points. On the other hand, Bergstrom and Carlson (1965) and Solyom *et al.* (1967), observed evidence favoring a stimulated fatty acid mobilization and triglyceride synthesis in adipose tissue from rats fed an essential fatty acid deficient diet, and suggested the possibility that prostaglandin synthesis may be decreased in tissues of the EFA-deficient rats. It will be recalled in this connection, that prostaglandins have been shown to act as inhibitors of lipolysis (Bergstrom *et al.*, 1964b), and it was assumed that a reduced level of prostaglandins in adipose tissue from EFA deficient rats permitted increased lipolysis. A similar suggestion was offered by De Pury and Collins (1965). Neither group of workers estimated tissue levels of prostaglandins, however, to determine whether there actually was a reduction in the tissue of EFA-deficient animals. Pawar and Tidwell (1968) have suggested that corn oil diets may promote the formation of greater amounts of prostaglandins from the unsaturated fatty acids ingested.

Prostaglandins E₁ and E₂ infused into the arterial supply were rapidly removed from the blood during a single circulation (Ferreira and Vane, 1967), suggesting that they might readily become depleted, with the disappearance of arachidonic acid, in an essential fatty acid-deficient state.

Ruddon and Johnson (1967) found only a slight effect of prostaglandins E₁ and F_{1a} on the synthesis of macromolecules in a cell-free system and concluded that if they function in intracellular control mechanisms, it may be at some other level of integration of cellular activity. It was suggested, also, that any effects these substances have on biosynthetic mechanisms may be evident only on certain target organs *in vivo*.

While most of the pharmacological effects of the prostaglandins do not relate these compounds to the gross manifestations of an essential fatty acid deficiency, certain observations suggest that one should continue to look at the prostaglandins as the possible active forms of the essential fatty acids. For example, the possibility that the contractile action of the prostaglandins is coupled with oxidative metabolism, and their possible influence on membrane function, discussed by

Bergstrom *et al.* (1968), suggests an explanation for alterations in oxidative mechanisms in liver mitochondria from essential fatty acid-deficient rats that have been described by Johnson and co-workers (Levin *et al.*, 1957; Johnson, 1963a,b; Ito and Johnson, 1964; Trojan and Johnson, 1968). This is in keeping with the suggestion that prostaglandins may not be circulatory hormones, but may act intracellularly as coenzymes or as factors in cellular control mechanisms (Horton, 1965).

I. ESSENTIAL FATTY ACID DEFICIENCY LESIONS

Deficient rats have an increased liver concentration of triglycerides and phospholipids. Serum fatty acids are increased; triglycerides and phospholipids are decreased. The composition and level of the plasma very low density lipoproteins is altered, but protein synthesis appears normal (Sinclair and Collins, 1968); with fat-free diets, however, there is an increased capacity for fatty acid synthesis (Reiser *et al.*, 1963b; Allman and Gibson, 1965).

EFA deficiency lesion studies previous to 1960 have been reviewed by Aaes Jorgensen (1961). EFA-deficient animals have an increased susceptibility to bacterial infections (Boyd and Edwards, 1966; Hopkins *et al.*, 1963; Stein *et al.*, 1968). Marion and Edwards (1962) and Menge (1968) and co-workers studied the effects of EFA on various aspects of egg production by hens. Their results may be summarized by indicating that an increase in egg production, egg size, fertility, and hatchability parallel an increase in dietary linoleate. Jenkins and Ewan (1967) found that selenium supplementation in a dystrophogenic diet (deficiency of tocopherol and sulfur amino acids) prevents dystrophy in lambs, calves, and pigs, but not chicks, unless linoleic acid is also removed from the diet. Linoleic acid, but not oleic or linolenic acid, inhibits the antidystrophogenic activity of selenium in chicks. The higher polyenoic acids 5,8,11,14,17-20:5 and 4,7,10,13,16,19-22:6 also precipitate myopathy. EFA-deficient Chinook salmon have an impaired pigmentation. Depigmentation also occurs with linolenic acid diets even though there is a positive growth response. Histochemical tests indicated an impaired melanin formation (Nicolaides and Woodall, 1965). Hands *et al.* (1965) found a decreased visual acuity in EFA-deficient rats. Spink and Su (1963) found that mice were protected against lethal amounts of exotoxins, endotoxins, and snake venom when the toxins or venom were mixed with oleate or linoleate prior to injection. Separate injections also gave protection. Melin Koff *et al.* (1965) reported some effects of dietary fat upon steroid and amino acid

metabolism. They fed normal adult men a high saturated fat-low polyenoic acid diet for 11 days followed by a low saturated-high polyenoic acid diet for 21 days. Changes observed following the second regimen were decreased plasma cholesterol, total lipid, leucine, and valine level; an increased proline, cystine, arginine, 3-methylhistidine, and aspartic acid level. There was also a decreased urinary excretion of total neutral 17-oxosteroids, 11-deoxo, 17-oxo steroids, etiocholanolone, cystine, valine, 3-methylhistidine, and β -alanine; an increased urinary excretion of anserine, cystathionine, and 1-methylhistidine. Naismith (1962) studied the role of EFA in protein utilization. Food consumption of EFA-deficient animals was 40-60% greater than normals. Nitrogen retention in EFA-deficient males was reduced 35%; and 41% in EFA-deficient females. The differences were reflected in changes in body weight. Conversion of 5-hydroxytryptophan to serotonin and oxidized 5-hydroxyindol metabolites are significantly lower with liver homogenates from rats fed low levels of polyenoic acids as compared with the activities of preparations from animals fed high levels of polyenoic acids. Monoamine oxidase and catecholorthomethyl-transferase activities in brain were unaffected by the dietary lipid (Century and Horwitt, 1967). Liver monoamine oxidase and 5-hydroxytryptophan metabolism were affected by dietary lipid (Century and Horwitt, 1968). Extensive degenerative changes occur in seminiferous tubules of EFA-deficient rabbits. Glucose-6-phosphate dehydrogenase and Δ^5 -3 β -hydroxy steroid dehydrogenase activity in Leydig cells was present in both fat-deficient and normal groups (Ahluwalia *et al.*, 1967). Caster and Ahn (1963) found a notching in the QRS complex of electrocardiograms in EFA-deficient rats. The QRS pattern change, indicating altered ventricular conduction, appeared before dermatitis occurred in the deficient rats. Addition of linoleic, linolenic, or arachidonic acid to the deficient diets caused the notching to disappear. Pigs fed fat-free diets develop severe dermal lesions (Sewell and McDowell, 1966), but these lesions have not been observed with pigs fed an EFA-deficient diet containing hydrogenated coconut oil (Babatunde *et al.*, 1967). Allt *et al.* (1969) have reported that EFA-deficient mature pigs treated with nicotine hydrogen tartrate showed no deficiency symptoms.

J. ESSENTIAL FATTY ACID METABOLITES: LIPID PEROXIDATION

Microsomal lipid peroxidation is a reaction in search of a function. Since *in vitro* peroxides damage a variety of protein functional groups (O'Brien and Frazer, 1966; Packer *et al.*, 1967; Barber and Bernheim,

1967) peroxidation has been associated uncritically with *in vivo* destructive processes. Polyenoic acids peroxidize rapidly in the presence of many biochemicals, e.g., heme pigments, but it is unlikely that oxygen tensions *in vivo* would support autoxidation. Hochstein and Ernster (1963) showed that microsomal peroxidation is supported by NADPH, thus indicating an enzymatic reaction. Wills (1969) has given evidence showing that lipid peroxidation is a function of microsomal electron transport. It appears highly improbable that the cell furnishes enzymes for the random destruction of its constituents.

Peroxidation proceeds by the same initial reaction sequence as lipoxidation and prostaglandin formation, the only reactions that are specific for the EFA structure. The initial reaction sequence for peroxidation is shown in Fig. 3 (see p. 133). Hydrogen is removed from the methylene bridge, yielding a highly reactive free radical intermediate [II]. The unpaired electron shifts, forming a radical-trans-cis system, which is then further oxidized to yield products. The free radical intermediate [II] is readily formed and reacts in microseconds (Tappel, 1962). Lipoxidation and prostaglandin formation involve stereospecific hydrogen removal. For example, lipoxidase incubated with 8,11,14-20:3 forms exclusively 15-L-hydroperoxy, c8,c11,t14-20:3 (Hamberg and Samuelsson, 1967a,b). In animal systems hydroperoxy acids are reduced to hydroxy acids by intracellular GSH-peroxidase (Little and O'Brien, 1968; Christoffersen, 1969; O'Brien and Little, 1969). Hydroxy acids have been isolated or implicated by several workers. May and McCay (1968) found that in the presence of NADPH microsomes enzymatically alter phospholipid polyenoic acids to form compounds with a primary absorption band at 230-232 m μ (absorption for conjugated hydroperoxide). Pace-Asciak and Wolfe (1968) isolated hydroxy acids from reactions using seminal vesicle and stomach acetone powders as the enzyme source. Swell and Law (1965) described the conversion of cholesteryl arachidonate to a highly polar lipid with the characteristics of a hydroxy acid. Hamberg and Samuelsson (1967b) isolated and characterized the hydroxy acids formed by incubating 8,11,14-20:3 with vesicular gland enzymes. Niehaus and Samuelsson (1968) have shown that arachidonate is the major source of peroxidation products (malonaldehyde) produced during microsomal lipid peroxidation.

For the purposes of further discussion, we assume a three-step model for membrane synthesis that assigns a function to microsomal lipid peroxidation consistent with structural requirements for EFA and established data concerning membrane reactions in EFA-deficient animals. The model proposes that microsomal lipid peroxidation func-

tions to control the release of phospholipids from their site of synthesis on the endoplasmic reticulum. Mitochondria are used as the target site requiring membrane lipoprotein. In step 1, mitochondria via normal aging or entropic consideration require new lipoprotein membrane. A message is sent to the endoplasmic reticulum to start lipid and protein synthesis. (Possibly this message is the product of the mitochondrial DNA. If so, then all cellular membranes should have their own DNA-RNA.) In step 2, the endoplasmic reticulum synthesizes the required lipid and protein. The lipid and protein are released in a manner that enables them to self-assemble to form the required lipoprotein. In step 3, the required lipoprotein is incorporated into the mitochondria. There are many ways in which hydroperoxide polyenoic acid derivative could react with the endoplasmic reticulum to control lipid release. Little and O'Brien (1968) have shown that at low concentrations the peroxide fatty acid moiety of methyl linoleate hydroperoxide stabilizes protein thio groups. The hydroperoxide could react directly with nucleic acids. In the absence of EFA, the correct hydroperoxide cannot form or the hydroperoxide formed does not accurately control lipid release.

The model assumes that the rate of cellular lipoprotein formation is the basic metabolic EFA lesion for the following reasons. Many phospholipid-requiring enzymatic reactions are known. The phospholipid requirement is nonspecific. Any phospholipid that can form the proper micelle is suitable (Jones *et al.*, 1969; Green and Tzagoloff, 1966).

There have been many suggestions that the absence of EFA in lipoprotein causes an unstable membrane. However, the most sensitive indicators of mitochondrial membrane function—respiration, oxidative phosphorylation, the capacity for energized translocation of mono-and divalent cations, ATPase activity—are unchanged by EFA deficiency (Ito and Johnson, 1964; Stancliff *et al.*, 1969). These functions are altered only when the mitochondria are stressed. This suggests that the EFA-deficient membranes are normal but “aged” or entropically downhill compared to membranes with EFA. An impaired rate of membrane formation would be the cause and is consistent with the observation of Bailey *et al.* (1967) that phospholipids in EFA-deficient mitochondria turn over more slowly than in normal mitochondria.

An alternative possibility is that peroxidation spontaneously occurs in membranes. The phospholipid hydroperoxide stabilizes the protein fraction of the membrane lipoprotein. This possibility is consistent with the suggestion of Collins *et al.* (cf. Section V) that arachidonate-protein interaction modulates membrane turnover, and offers

a partial explanation for the existence of individual phospholipid molecular species (Section IV, C). Also, EFA-deficient mitochondria are more resistant to lipid peroxidation (Stancliff *et al.*, 1969).

K. DIET AND TISSUE POLYENOIC ACID CONTENT

The literature concerning changes in tissue lipid content with changes in dietary lipids is extensive. Carroll (1965) has written an excellent discussion on the effects of dietary fat on tissue lipid composition in various animals. References on EFA deficiency are listed in Table VI with some references on normal tissue fatty acid composition. With few exceptions, e.g., Leghorn pullet pituitary gland (Menge, 1967), and New Zealand rabbit seminal vesicle (Ahluwalia *et al.*, 1967), all studies have confirmed that EFA tissue content decreases while non-EFA (oleic and palmitoleic acid metabolites) tissue content increases when animals are fed EFA-deficient diets. This pattern is quickly reversed by the addition of EFA to the deficient diet. Tissue EFA depletion or recovery rates are different for each tissue and may be a function of each tissue (Walker, 1967a, 1968).

A puzzling aspect of polyenoic acid metabolism is the lack of regularity in the pattern of polyenoic acid metabolites. Linoleic acid is converted to arachidonic acid, but only very small levels of the intermediate acids, e.g., 6,9,12-18:3 and 8,11,14-20:3, are present in tissues. Marcel *et al.* (1968) pointed out that the desaturation of 8,11,14-20:3 is strongly enhanced by 9,12-18:2 and 6,9,12-18:3, which may account for its nonaccumulation in tissue lipids. Animals fed linolenic acid have very low tissue levels of linolenic acid, 11, 14, 17-20:3, or 8,11,14,17-20:4; EFA-deficient animals have high tissue levels of 5-, 8,11,20:3, but almost no 6,9-18:2; 3,6,9-18:3, or 8,11-20:2. Eighteen-carbon acids with three double bonds do not appear to accumulate in tissues under any conditions. Apparently, in agreement with the results of Marcel *et al.*, these acids are favored substrates for extension and olefination. The reason why only certain 20-carbon acids with three or four double bonds accumulate is less clear, especially in view of the fact that 8,11,14-20:3 is a substrate for prostaglandin synthesis.

No correlation has been found between EFA deficiency lesions and tissue fatty acid composition, yet a common hypothesis persists in EFA research: the presence of nonessential polyenoic acids in membranes affords an unstable lipoprotein membrane. It is possible that membrane proteins will combine with any lipid that can achieve a conformation suitable for protein combination. Such a conformation would be dependent upon the quantity, not the type, of fatty acid un-

Table VI
DIET AND TISSUE LIPID CONTENT

Tissue and animal	Diet ^a	Analysis ^b	Reference
<i>I. Blood plasma</i>			
1. Male rat	Fat free 1.0 ml safflower oil	Samples at 0-24 hours, PL and cholesterol	Johnson <i>et al.</i> (1967)
2. Pigs	0.07-3.7% cal. linoleate	Serum total FA CE, PL, TG Free FA Total FA	Leat (1963)
3. Monkey	Fat free Normal 7% stearic acid 1 gm cholesterol plus	Serum CE, PL, TG	Greenberg and Moon (1961)
4. Male rabbit	1 gm olive oil 150 mg linoleate 150 mg arachidonate 10% safflower oil 10% coconut oil	CE, PL, TG CE	Swell <i>et al.</i> (1961)
5. Male and female rats	Normal 1 gm cholesterol plus 8% olive oil 8% corn oil	CE, PL, TG	Okey <i>et al.</i> (1962)
6. Male rabbit	Fat free 15% cal. lard	CE	Swell <i>et al.</i> (1962)
7. Beagle puppies	Fat free 10% corn oil	Total FA	Patil and Hansen (1962)
8. Male rat	10% lard 10% menhaden oil	TG, PL	Privett <i>et al.</i> (1965)

(Continued)

Table VI (Continued)

Tissue and animal	Diet ^a	Analysis ^b	Reference
9. Rat	1 ml coconut oil 1 ml olive oil 1 ml cod liver oil	Chyle serum and chylomycron FA	Bragdon and Karmen (1960)
Human female	2 gm/kg body weight corn oil 2 gm/kg body weight coconut oil	Serum chylomycron FA	
10. Female rat	15% coconut oil 15% sunflower seed oil	Serum FA	Rathbone (1965)
<i>II. Blood Cells</i>			
11. Male rat	0.2% cal. corn oil 15% cal. corn oil 15% cal. coconut oil 7% cal. cod liver oil Fat free 8% lard plus 3% cod liver oil	Erythrocyte FA	Witting <i>et al.</i> (1961)
12. Rhesus monkey	10% hydrogenated coconut oil Cf. 3	Erythrocyte FA	Fitch <i>et al.</i> (1961)
13. Male rat	10% hydrogenated coconut oil	Erythrocyte FA	Walker (1966)
14. Rhesus monkey	Cf. 3	Erythrocyte FA	Greenberg and Moon (1961)
15. Beagle puppies	Cf. 7	Erythrocyte FA	Patil and Hansen (1962)
16. Male rat	10% corn oil 10% lard	Erythrocyte stroma choline and non-choline PL	Walker and Kummerow (1963)
17. Male rat	Fat free 0.009-4.87% cal. linoleate 0.007-3.75% cal. linolenate 0.009-9.42% cal. arachidonate	Erythrocyte FA	Mohrhauer and Holman (1963b)

18.	Male and female humans	16 days fat free 45% cal. corn oil 45% cal. butterfat	Erythrocyte CE, PL, TG	Hill <i>et al.</i> (1964)
19.	Male human	16 days fat free 40% corn oil 40% butterfat	Erythrocyte fractionated PL	Hill <i>et al.</i> (1965)
20.	Male and female humans	Normal 8 wks. 40% linoleate 15 wks. 40% triolein Fat free 15% corn oil 15% hydrogenated coconut oil	Erythrocyte choline FA Leukocyte FA PL and non-PL of granular fraction	Faruquier (1965) Yu <i>et al.</i> (1966)
21.	Male rat	Fat free 10% linoleate Fat free 10% linoleate Fat free 0.8% corn oil 20% corn oil 20% butterfat 24% butter 20% hydrogenated coconut oil 5% cal. hydrogenated coconut oil 33% cal. hydrogenated coconut oil 33% cal. corn oil Fat free 1 ml safflower oil	Total FA 40 μ J/day linoleic-family FA 40 μ J/day linolenic-family FA Total FA Choline FA FA in the 1 and 2 position of ethanol- amine, choline, and TG	Rahm and Holman (1964b) Jensen <i>et al.</i> (1968) Collins (1966) Moore and Williams (1965)
III.	Liver		Choline FA	
22.	Male rat	Fat free 10% linoleate Fat free 10% linoleate Fat free 0.8% corn oil 20% corn oil 20% butterfat 24% butter 20% hydrogenated coconut oil 5% cal. hydrogenated coconut oil 33% cal. hydrogenated coconut oil 33% cal. corn oil Fat free 1 ml safflower oil	Choline FA Van Golde and Van Deenen (1966)	
23.	Male rat			
24.	Male rat			
25.	Male rabbit			
26.	Male rat			
27.	Male rat			From 0 to 24 hours CE, TG, fraction- ated PL
				Johnson <i>et al.</i> (1967)

(Continued)

Table VI (Continued)

Tissue and animal	Diet ^a	Analysis ^b	Reference
28. Male rat	Fat free 33% cal. corn oil	Choline FA	Van Goldé <i>et al.</i> (1968)
29. Female rat	Fat free 0.01-4% cal. linoleate	Total FA	Pudlakiewicz <i>et al.</i> (1968)
30. Male rat	0.01-4% cal. linolenate 5% coconut oil 5% dalda 5% ghee	CE, PL, and TG polyenoic acids	Patil and Magar (1960)
31. Male and female rats	5% ground nut oil with and without cholesterol	CE, PL, and TG polyenoic acids	Morton and Horner (1961)
32. Male rat	Fat free 100 mg/day linoleate	Total FA	Biran <i>et al.</i> (1964)
33. Male rabbit	Normal 100 mg/day linseed oil	CE, PL, TG	Swell <i>et al.</i> (1961)
34. Male and female rats	Cf. 4 Cf. 5	CE, PL, TG	Okey <i>et al.</i> (1962)
35. Male rabbit	Cf. 6	CE	Swell <i>et al.</i> (1962)
36. Swine	0-12% cal. linoleate	Polyenoic acids	Caster <i>et al.</i> (1962)
37. Male rat	Fat free 50-100 mg/day of linoleic and linolenic-family FA alone and in combination	Total FA	Rahn and Holman (1964a)
38. Male rat	Cf. 8	PL, TG	Privett <i>et al.</i> (1965)

39. Male rat	Cf. 17	Total FA	Mohrhauer and Holman (1963a)
40. Male rat	Cf. 22	Total FA	Rahn and Holman (1964b)
41. Male rat	Fat free	Total FA	Casal and Holman (1965)
	With or without 1% eal.		
	Linoleate		
	74% sucrose		Ostwald <i>et al.</i> (1965)
	74% glucose		
	74% maltose		Catala and Brenner (1967)
	74% starch	CE, PL, TG	
42. Male and female rats	Safflower oil	PL, TG	
	Hydrogenated coconut oil		
43. Male rat	Fat free	PL, TG	
	100 mg/day linoleate		
	400 mg/day linoleate		
IV. Kidney			
44. Male rat	Cf. 32	Total FA	Biran <i>et al.</i> (1964)
45. Male rat	Cf. 8	PL, TG	Privett <i>et al.</i> (1965)
V. Heart			
46. Male rat	Cf. 22	Total FA	Rahn and Holman (1964b)
47. Male rat	Cf. 32	Total FA	Biran <i>et al.</i> (1964)
48. Male rabbit	Cf. 4	Aorta CE, PL, TG	Swell <i>et al.</i> (1961)
49. Male rabbit	Cf. 6	Aorta CE	Swell <i>et al.</i> (1962)
50. Swine	Cf. 36	Total polyenoic acids	Caster <i>et al.</i> (1962)

(Continued)

Table VI (Continued)

Tissue and animal	Diet ^a	Analysis ^b	Reference
<i>VI. Brain</i>			
51. Female rat	Cf. 10	Brain and Myelin total FA	Rathbone (1965)
52. Rats, 0-3 weeks old	Maternal diet Normal 10% corn oil	Total FA	Walker (1967a)
<i>VII. Intestine</i>			
53. Male rat	Fat free 120 mg/day linoleate with or without antibiotics 120 mg/day arachidonate	Intestinal mucosa, contents and fecal lipids FA	Bottino (1967)
54. Male rat	Fat free Normal Starved 24 hours 100 mg/day linoleate Cf. 32	Proximal and medial ileum, muscle and mucosa, colon muscle and mucosa	Ensor and Bartley (1962)
55. Male rat	Cf. 22	Total FA	Biran <i>et al.</i> (1964)
<i>VIII. Testes</i>			
56. Rat	Fat free Normal	Total FA	Rahn and Holman (1964b) Ahluwalia <i>et al.</i> (1967)
57. New Zealand rabbit		Total FA of testes and seminal vesicles	Jensen <i>et al.</i> (1968)
58. Rat	Cf. 23	CE, PL, TG Glycerol ethers and diglycerides	Sewell and Miller (1966)
59. Pig	Fat free 0.25-4.00% cal. Linoleate	Total FA	

60. Pig	Cf. 59	Total FA	Sewell and McDowell (1966)
61. Rat	Cf. 8	Epididymal TG	Privett <i>et al.</i> (1965)
62. Rat	Cf. 22	Total FA	Rahn and Holman (1964b)
<i>IX. Adrenals</i>			
63. Male rabbit	0.21-10.6% linoleate	CE	Moore and Williams (1966)
<i>X. Liver mitochondria</i>			
64. Male rat	Cf. 22	PL and non-PL	Rahn and Holman (1964b)
65. Male rat	Fat free 5% corn oil	Fractionated PL 1-and 2-positioned FA	Johnson and Ito (1965)
66. Male rat	Fat free	Fractionated PL	Tischer and Glenn (1965)
	50-100 mg/day linoleate		
	50-100 mg/day linolenate		
67. Male rat	Fat free 7% linoleate	Fractionated PL	Yamamoto <i>et al.</i> (1965)
68. Male rat	7% linolenate	Neutral lipids	Biran <i>et al.</i> (1965)
69. Male rat	Normal 100 mg/day linseed oil Cf. 1	PL and fractionated PL Total FA	Witting <i>et al.</i> (1961)
<i>XI. Brain mitochondria</i>			
70. Female rat	Cf. 10	Total FA	Rathbone (1965)
71. Male rat	Cf. 1	Total FA	Witting <i>et al.</i> (1961)
<i>XII. Microsomes</i>			
72. Male rat	Cf. 67	Fractionated PL	Yamamoto <i>et al.</i> (1965)

(Continued)

Table VI (Continued)

Tissue and animal	Diet ^a	Analysis ^b	Reference
<i>XIII. Sarcotubular testicle</i>			
73. Male rat	Fat free 8% corn oil	Total FA	Yu <i>et al.</i> (1968)
<i>XIV. Adipose tissue</i>			
74. Mice	Fat free 1-50% safflower oil 1-50% glycerol monoleate 1-50% olive oil 1-20% oleate	Total FA	Tove and Smith (1960)
75. Male pig	Cf. 60	Total FA	Sewell and McDowell (1966)
76. Pig	Cf. 2	Total FA	Leat (1963)
77. Male and female rat	10% safflower oil 10% coconut oil	Subcutaneous mesenteric and interscapular total FA	Ostwald <i>et al.</i> (1962)
78. Male rat	Cf. 39	Total FA	Mohrhauer and Holman (1963a)
79. Male rat	Cf. 43	Epididymal, epirenal, and intestinal total FA	Catala and Brenner (1967)
<i>XV. Muscle</i>			
80. Male rat	Cf. 32	Total FA	Biran <i>et al.</i> (1964)

XVI. Fish				
81. Rainbow trout	10% corn oil 5% salmon oil, 5% corn oil 1% salmon oil, 9% corn oil 10% soybean oil 7.8% corn oil 10% palmitate 10% oleate 10% linoleate Fat free	Whole fish	Lee <i>et al.</i> (1967)	
82. <i>Pimelodus maculatus</i> <i>parapimelodus valenciennesi</i>	5% linoleate 5% linoleate 5% linoleate 5% linoleate 30% cotton seed oil 30% linseed oil	Whole fish	Brenner <i>et al.</i> (1963)	
83. Mullet <i>Fundulus</i> Goldfish	Careass total FA Goldfish TG and PL	Careass total FA Goldfish TG and PL	Reiser <i>et al.</i> (1963a)	
XVII. Birds				
84. Hen	Linoleate Linolenate Fat free 7% corn oil Corn-soy diet Normal diet	Egg total FA Plasma, heart, liver, bile, pancreas, kidney, spleen, ovary, brain, pituitary, pineal, thyroid, adrenal total FA	Murty and Reiser (1961) Menge (1967)	
85. White Leghorn pullets	0.016-5.0% eal. linoleate 12% tallow Fat free 12% tallow 12% linoleate 12% linolenate	Liver total FA Liver and cerebellum total and mitochondri- al FA	Hill (1966) Marco <i>et al.</i> (1961)	
86. Male Leghorn chicks				
87. Nichols 108 chicks				

(Continued)

Table VI (Continued)

Tissue and animal	Diet ^a	Analysis ^b	Reference
88. Nichols 108 cockerels	Fat free 5% myristate 3% laurate 2% safflower oil 2% cholesterol	Total liver depot fat FA Heart, testes and cerebrum total FA	Machlin and Gordon (1961)
89. White Leghorn pullets	Fat free 10-250 mg/day linoleate	Plasma and egg yolk total FA	Menge <i>et al.</i> (1965a,b)
90. Chicks	Fat free Normal 10% cotton seed oil 10% linseed oil 10% rapeseed oil 10% corn oil margarine 10% butterfat	Liver and bile fractionated PL Non-PL	Glehn and Dam (1965)
91. Chicks	Fat free 2% safflower oil	Carcass total FA	Machlin (1961)
92. White Leghorn male	Fat free 1 and 4% linoleate	Blood plasma and seminal total FA	Lillie and Menge (1968)
93. Thornber 606 hens	Fat free 8% corn oil	Egg total FA Egg TG and choline and ethanolamine PL	Balmave (1968)

^aOnly the lipid content of the diet is listed. "Normal" indicates standard laboratory chow. Where the authors have analyzed multiple tissues, reference to the first diet listing is given. "Cal." indicates weight percent of the lipid in terms of dietary calories.
^bCE, PL, TG indicate analysis of cholesterol ester, phospholipid, and triglyceride fatty acid (FA) content, respectively. Fractionated PL indicates the fatty acid analysis of the individual phospholipid species.

saturation. In support of this possibility Guarneri and Johnson (unpublished results) have shown that under certain conditions mitochondrial reactions that have been associated with EFA deficiency occur with a normal mitochondrial fatty acid content and the reverse: normal mitochondrial reactions take place in mitochondria having EFA-deficient-type fatty acid content. These experiments are described below.

Several mitochondrial reactions are altered in EFA deficiency: succinate, β -hydroxybutyrate, and NADH oxidation. Assuming that these altered mitochondrial reactions are characteristic of an EFA deficiency the following experiment was designed to determine how these reactions depend on mitochondrial lipid composition. Normal adult rats were starved 2 days then fed restricted levels of normal or EFA-deficient diets for 16-22 days. At the end of 16 days rats fed normal and deficient diet were sacrificed. Liver mitochondrial succinate, β -hydroxybutyrate, α -ketoglutarate, and hypotonic NADH oxidation rates were measured. Weight changes were followed and mitochondrial fatty acid composition was determined. Remaining rats on normal diets were kept on the restricted ration for an additional 6 days, then examined as were rats of the first set.

The results of this experiment are shown in Table VII. Fatty acid analysis data have been condensed by expressing the polyenoic acid and EFA content of the mitochondria as single values. Significant differences between the two values are due to a decrease in linoleic acid family fatty acids and an increase in palmitoleic and oleic acid family fatty acids. Rats fed a restricted normal diet (corn oil) for 16 days have lost an average of 102 gm, but their mitochondrial fatty acid pattern and NADH/state 3 oxidation ratio (see Section V) are normal. Succinate, β -hydroxybutyrate, and hypotonic NADH oxidation rates are similar to values found for normal rats. There was no change in α -ketoglutarate oxidation rates in any experiment. Rats fed a restricted deficient diet for 16 days have a 130-gm average weight loss, a deficient fatty acid pattern, but a NADH/state 3 oxidation ratio of 0.46. Rats fed a restricted normal diet for 22 days have a 135-gm average weight loss, a normal fatty acid pattern but a NADH/state 3 oxidation ratio of 0.25. Clearly, rats can have a deficient fatty acid pattern with normal NADH/state 3 values and a normal fatty acid pattern with deficient NADH/state 3 values. Values for succinate and β -hydroxybutyrate oxidation are in good agreement with the results of Trojan (1966), who showed that intact EFA-deficient mitochondria had significantly higher succinic and β -hydroxybutyric oxidase activity com-

Table VII
MITOCHONDRIAL REACTIONS AND FATTY ACID CONTENT OF RATS ON RESTRICTED DIETS^a

Diet, 5 gm/day/rat	Polyenoic acid content ^b (% of total fatty acids)		Mitochondrial oxidation reactions ^c (μ moles O ₂ per mg protein)			
	Total	EFA	Succinate	β -Hydroxybutyrate	NADH	NADH/state 3
5% corn oil diet for 16 days	42 ± 3	39 ± 2	62 ± 20 (4.5 ± 0.3)	27 ± 7 (4.5 ± 0.2)	27 ± 8	0.44 ± 0.02
Fat-free diet for 16 days	38 ± 4	15 ± 4	69 ± 18 (4.3 ± 0.3)	24 ± 6 (3.6 ± 0.2)	30 ± 3	0.46 ± 0.01
5% corn oil diet for 22 days	41 ± 4	40 ± 3	73 ± 7 (3.8 ± 0.4)	34 ± 2 (3.6 ± 0.3)	19 ± 2	0.25 ± 0.02

^aAll values are expressed as the average of 3 experiments ± SD.

^bPolyenoic acid is the total of all acids having 2 or more double bonds. EFA is the total of 18:2 ω 6, 20:4 ω 6, and 22:4 ω 6 fatty acids.

^cValues for succinate and β -hydroxybutyrate are expressed as State 3 oxidation of substrate. Numbers in parentheses indicate respiratory control values. NADH oxidation is measured in hypotonic solution.

pared to normal mitochondria; respiratory controls in normal and deficient mitochondria were the same with succinate, but significantly lower in the deficient preparations when β -hydroxybutyrate was substrate.

Regarding mitochondrial reactions the similarities between rats fed restricted normal diet and EFA-deficient diet suggests a similarity in lesions. Mitochondria (half-life = 4-6 days for proteins) are in a dynamic state (Swick *et al.*, 1968) and must require a constant supply of structural materials. An inefficient supply of structural material through a restricted diet or an EFA-deficient diet would impair mitochondrial function. This implies that EFA-deficient animals are capable of synthesizing only an adequate supply of suitable phospholipid micelles for membrane function. The more rapid deterioration under stress of EFA-deficient membranes compared to normals reflects the biosynthetic inability of EFA-deficient animals to efficiently satisfy dynamic membrane requirements.

V. Essential Fatty Acids and Membrane Function

It has been recognized for some time that membranes are active participants in many biological processes, but only recently have precise mechanisms for this been elucidated. This has come about largely as a result of the physiochemical characterization of some of the membrane structures, and more particularly with the realization that certain organelles, viz. mitochondria, lysosomes, and microsomes, are membranous in nature. It now seems likely that (1) the electron transport activity of mitochondria is intimately associated with a membrane structure, (2) the membranous phospholipids are essential to this activity, (3) probably all integrated functions of mitochondrial inner membrane, at least, are lipid dependent, and (4) the nature of the fatty acid moiety of the phospholipids is of importance.

This portion of the review draws attention to studies on the biochemistry and physiology of the essential fatty acid deficient state, as these may be concerned in membrane function. Most of the evidence emerging at this time suggests that the essential fatty acids function as moieties of one or another of the phospholipids which are an integral part of active membranes. A number of excellent reviews have recently appeared which describe and relate membrane structure and function, to which the reader is referred. The New York Academy of Sciences has provided an extensive review of the biology of membranes, including membrane biochemistry and biophysics, in a recent issue of the *Annals* (Meyer, 1966). Other reviews are those by Green

and Tzagoloff (1966), Van Deenen (1965), and Aaes Jorgensen (1961). The latter two deal to a greater degree with the essential fatty acids than the others.

The earliest suggestion that an EFA-deficient rat may possess "abnormal membranes" was made, without much supporting evidence at the time, by Burr and Burr (1930). Since that time a number of investigations have provided support for this idea, and suggested that lipoprotein membranes in EFA-deficient rats are relatively less stable than those in normal animals (Klein and Johnson, 1954; Tulpule and Williams, 1955; Levin *et al.*, 1957; Hayashida and Portman, 1960a,b; De Pury and Collins, 1963; Johnson, 1963a; Wilson and Leduc, 1963; Ito and Johnson, 1964; Stein and Stein, 1964), and that alteration of these membranes as to structure and permeability is at least one of the results of an EFA deficiency.

Altered membranes may account for some of the skin changes (Ramaingeswami and Sinclair, 1951; Parros and Finerty, 1954; Funch *et al.*, 1957), susceptibility to infections (Ross and Adamson, 1961; Hopkins *et al.*, 1963; Menge *et al.*, 1965b), capillary fragility (Kramar and Levine, 1953), and high rate of transepidermal water loss (Menton, 1966) seen in EFA-deficient animals.

Perhaps the most direct evidence that lipoprotein membranes may be altered in an EFA deficiency is provided by studies on mitochondrial swelling *in vitro*. Liver mitochondria prepared from EFA-deficient rats swell rapidly *in vitro* under conditions that preserve the shape and size of normal mitochondria (Hayashida and Portman, 1960b) and, indeed, may even be swollen *in vivo* (Levin *et al.*, 1957; Smithson, 1967; Wilson and Le Duc, 1963). Hayashida and Portman observed that ATP prevented the swelling of normal mitochondria but not of EFA-deficient mitochondria in 0.30 M sucrose; Johnson (1963a) reported that ATP reversed spontaneous swelling of EFA-deficient mitochondria in 0.154 M KCl as well as swelling induced by Ca^{2+} and thyroxine, to a greater degree than in normal mitochondria, and that, in the presence of digitonin, deficient mitochondria swelled more rapidly than normal ones (Trojan and Johnson, 1968).

A dependence of membrane integrity on dietary fat was suggested also by Century and Horwitt (1963), who found that when rats were fed corn oil or cod liver oil the liver mitochondria swelled more in the presence of silver ions and less in the presence of thyroxine than when beef fat was fed. Also, liver mitochondria from chicks fed corn oil were more sensitive to the swelling effects of silver, copper, and mercuric ions than those from chicks fed lard.

A possible explanation for the apparent lability of EFA-deficient membranes was offered by Collins (1962), who noted that ^{32}P was incorporated into the lecithins and phosphatidylethanolamines of liver faster in EFA-deficient rats than in normal ones, and that the slower incorporation into lecithins occurred in the fraction containing arachidonic acid (Collins, 1960, 1963). Arvidson (1968) has confirmed these data. On the basis of these observations, Collins suggested that arachidonic acid, presumably because of the hooked shape of its hydrocarbon tail, serves as a modulator of phospholipid metabolism and contributes to an increased structural stability of membranes by its presence as part of the membrane phospholipid. However, Bailey *et al.* (1967) found that phospholipid in mitochondria from EFA-deficient rats turnover more slowly (half-lives = 3 and 29 days) compared to phospholipids from normal mitochondria (half-lives = 1.6 and 10 days). In a subsequent investigation, De Pury and Collins (1966) studied the influence of fatty acid composition of lecithin obtained from normal and EFA-deficient rats on the rate of its binding to liver mitochondrial protein. They suggested that hydrophobic bonds between structural proteins and phospholipids are influenced by the nature of the fatty acid moieties of the phospholipids. The normal rat phospholipids bound the proteins more slowly than did phospholipids from EFA-deficient rats, and these authors suggested that special sites exist for the hydrophobic bonding of arachidonic acid, such that phospholipids containing it are bound more slowly but more firmly than other phospholipids.

This is in harmony with the suggestion (Turpeinen, 1937; Sinclair, 1952) that the symptoms of a deficiency of the essential fatty acids were due to a lack of arachidonic acid, and the observations of Rahm and Holman (1964b) that rats fed a fat-free diet supplemented with arachidonic acid failed to develop signs of an EFA deficiency, even though there had been no conversion of arachidonic to linoleic acid in mitochondrial lipids. It is also in the keeping with the observation (Johnson and Ito, 1965) that mitochondrial phospholipids of rats fed a fat-free diet are rapidly depleted of arachidonic acid.

There have been reports of lability of other organelle membranes associated with an EFA deficiency. For example, MacMillan and Sinclair (1958) reported EFA-deficient erythrocytes to be more liable to osmotic lysis than normal ones. Trojan and Johnson (1968), however, reported that both erythrocytes and liver mitochondria from EFA-deficient rats were more resistant to the swelling effect of cupric ion than were those prepared from rats fed a corn oil diet. Their observa-

tions might be explained if it is assumed that the lamella phospholipid-protein reaction in an EFA-deficient animal results in a relatively decreased accessibility of protein sulfhydryl groups to metal ions, e.g., Cu^{2+} .

In view of the phospholipid content of lysosomal membranes (Tappel *et al.*, 1965), Moore *et al.* (1967) made a study of digestive activities of lysosome preparations from normal and EFA-deficient rats; they observed that while there were differences between the normal and deficient preparations, the changes in hydrolytic activity and membrane stability did not seem sufficient to explain the lesions of an EFA deficiency.

Gerschenson *et al.* (1967) observed that pulsating rat heart cells, when cultured in a lipid-deficient medium, showed some of the symptoms of an EFA deficiency, viz. lack of growth, impairment of oxidative phosphorylation, decrease in respiratory control, and a characteristic fatty acid pattern. The addition of albumin-bound linoleic or arachidonic acid prevented partially or totally the impaired mitochondrial function and fatty acid pattern. Albumin-bound palmitic acid did not prevent these effects, suggesting that the effects on oxidative phosphorylation and respiratory control, ascribed by Johnson *et al.* (Levin *et al.*, 1957; Johnson, 1963b; Ito and Johnson, 1964), deficiency of the whole animal, had indeed been produced in cell culture. Interestingly, however, Gerschenson *et al.* (1967) concluded on the basis of their data that, while fatty acids seem to be involved in heart cell maintenance and the essential fatty acids are concerned in mitochondrial function, their involvement in cell maintenance is not through their function in mitochondrial membranes.

Van Deenen (1965) reported that a high degree of parallelism existed between the decreases in palmitic acid:oleic acid ratios (Kogl *et al.*, 1960), lecithin:sphingomyelin ratios (DeGier and Van Deenen, 1961), loosely bound phospholipid (Van Deenen, 1965), and the decrease in permeability of various mammalian erythrocytes toward penetrating nonelectrolytes, such as glycerol and urea (Jacobs *et al.*, 1950). It was also noted that decrease in the ability of *Crotalus adamanteus* venom to hydrolyze the phosphoglycerides of red cell lysates followed the same species order. Thus chemical variations in the phospholipids contribute measurably to variations in the properties of biomembranes other than the mitochondria.

A number of biochemical changes have been described in mitochondria from EFA-deficient animals, but direct evidence is lacking to prove them unique to the deficiency, or to indicate that they are indeed the site where the essential acids exert their essentiality.

There can be little doubt, however, that circumstantial evidence implicates the essential fatty acids—at least arachidonic acid—in maintenance of the morphological integrity of the mitochondrial membrane, and in the proper functioning of certain oxidative and related mechanisms that depend for integrated function on an intact membrane structure. Further, the evidence indicates that the essential fatty acids act, in this role, as components of some of the phospholipids.

Klein and Johnson (1954) reported an uncoupled oxidative phosphorylation in liver mitochondria from EFA-deficient rats, and Levin *et al.* (1957) confirmed this, noting that deficient mitochondria were swollen, and that normal mitochondria could be made to resemble deficient ones in phosphorylating and certain oxidative capacities simply by lowering the osmolarity of the suspending medium. Under this circumstance, the normal mitochondria would be expected to swell. Moore *et al.* (1969) noted that the rate of Na^+ efflux from within artificial lecithin membranes was a function of the lecithin source. Activation energies for Na^+ efflux were 15, 11.5, 9.5, and 4.5 kcal of Na^+ per mole for lecithins isolated for rats fed normal diets (44% $\omega 6$; 2% $\omega 3$ fatty acid content): fish (16% $\omega 6$; 39% $\omega 3$); egg (30% $\omega 6$; 1% $\omega 3$) and EFA-deficient rat (5% $\omega 6$; 1% $\omega 3$), respectively.

In a subsequent study on the swelling tendency of EFA-deficient rat liver mitochondria, referred to earlier, Johnson (1963a) suggested that an (ATP- Mg^{2+})-mitochondrial relationship existed in normal mitochondria that did not occur in deficient ones, and further that the marked swelling tendency in EFA-deficient mitochondria was associated with altered respiratory and/or phosphorylating mechanisms (Johnson, 1963b).

Ito and Johnson (1964) observed that deficient mitochondria exhibit less stable respiratory control than normal preparations in the presence of digitonin, *C. adamanteus* venom, and Ca^{2+} and suggested a relatively labile membrane organization for the former. These workers later observed (Ito and Johnson, 1968) that in a hypotonic medium EFA-deficient liver mitochondria oxidized exogenous NADH much more slowly than did normal ones. When the two mitochondrial preparations were disrupted by sonication, they carried out the oxidation at similar rates, indicating that the deficient mitochondria had a normal complement of the respiratory chain components. It was concluded that a defect exists in the mitochondrial membrane of the EFA deficient rat, which was responsible for the differences in NADH oxidation under hypotonic conditions. Stancliff *et al.* (1969) found that, in general, the activities of the inner mitochondrial membrane, respiration, oxidative phosphorylation, the capacity for ener-

gized translocation of mono- and divalent cations, were largely unchanged by EFA deficiency. Electron microscopy indicated EFA-deficient mitochondria were larger *in situ*.

β -Hydroxybutyric dehydrogenase of beef heart mitochondria has been shown to have a unique requirement for lecithin, whose activity depends on the nature of the fatty acid moiety (Jurtshuck *et al.*, 1961; Sekuzu *et al.*, 1963). It is quite possible that extensive changes in fatty acid composition alter mitochondrial enzyme activities. Indeed, alterations in cytochrome oxidase (Tulpule and Williams, 1955), and succinic, butyric, and glutamic dehydrogenase activities (Tulpule and Patwardhan, 1952) have been observed in an EFA deficiency in rats.

In summary, the involvement of phospholipids in membrane structure and function, the presence of the essential unsaturated fatty acid in the phospholipids in normal liver mitochondria and their disappearance with the onset of an EFA deficiency, the demonstration by Collins *et al.* of the possible role of arachidonic acid in the binding of phospholipids to membranes, and the changes in membrane-related respiratory-linked biochemical reactions in EFA-deficient mitochondria, form the substance of evidence associating the biochemical role of the essential fatty acids with membrane function. Whether this can account for the gross manifestations of an essential fatty acid deficiency remains to be demonstrated.

VI. Summary

The role of the essential unsaturated fatty acids in the animal economy is not clearly defined. Obviously these acids are metabolized in ways common to other unsaturated fatty acids. In addition, the unsaturated acids possessing the 9, 12 double bond system (C_{18} acids) or the 11-14 double bond system (C_{20} acids) possess special properties that are essential to the animal. Presumably they function in esterified forms rather than as free fatty acids, and as such their essential roles may be determined by relative concentrations of phospholipids, glycerides, and sterol esters, which in turn seem to involve hormonal regulation.

There is evidence to suggest that they are concerned, via phospholipids, in membrane integrity. This may be related to certain biochemical lesions that have been reported in connection with an EFA deficiency in cases where membranes possess recognized biochemical activity, e.g., in mitochondria. In other tissues altered membranes may account for such gross morphologic lesions as the dermal symptoms described. Direct relationships have not been demonstrated in either case.

The essential fatty acids are the precursors of the prostaglandins, but the relationships of the latter to the classical deficiency symptoms is not certain, nor is there evidence suggesting a relationship of this hormone family to the reported biochemical lesions of the deficiency. Furthermore, it cannot be excluded that some of the symptoms are a manifestation of toxicity associated with metabolic products that accumulate during a deficiency.

References

- Aaes Jorgensen, E. (1961). *Physiol. Rev.* **41**, 1.
Aaes Jorgensen, E. (1966). *Nutr. Rev.* **24**, 1.
Aftergood, L., and Alfin-Slater, R. (1965). *J. Lipid Res.* **6**, 287.
Aftergood, L., and Alfin-Slater, R. (1967). *J. Lipid Res.* **8**, 126.
Ahluwalia, B., Pincus, G., and Holman, R. T. (1967). *J. Nutr.* **92**, 205.
Ahluwalia, B., Shima, S., and Pincus, G. (1968). *Fed. Proc. Abstr.* **3379**.
Alfin-Slater, R., and Aftergood, L. (1968). *Physiol. Rev.* **48**, 758.
Alfin-Slater, R., Morris, R. B., Hansen, H., and Procter, J. F. (1965). *J. Nutr.* **87**, 168.
Allman, D. W., and Gibson, D. M. (1965). *J. Lipid Res.* **6**, 51.
Allt, W. A., Pilkington, T. R. E., and Woolf, N. (1969). *Science* **163**, 391.
Anderson, R. (1968). *Biochim. Biophys. Acta* **152**, 531.
Anderson, R., and Coots, R. H. (1967). *Biochim. Biophys. Acta* **144**, 525.
Anderson, R., and Reiser, R. (1966). *Lipids* **1**, 233.
Anggard, E., Matschinsky, F. M., and Samuelsson, B. (1969). *Science* **163**, 479.
Arvidson, G. A. E. (1968). *European J. Biochem.* **5**, 415; 4 478.
Babatunde, G. M., Pond, W. B., Krook, L., Van Vleck, L. D., Walker, E. F., and Chapman, P. (1967). *J. Nutr.* **92**, 293.
Bailey, E., Taylor, C. B., and Bartley, W. (1967). *Biochem. J.* **104**, 1026.
Balnave, D. J. (1968). *J. Sci. Food Agr.* **19**, 266.
Barber, A. A., and Bernheim, F. (1967). *Advan. Gerontol. Res.* **2**, 355.
Beerthuis, R. K., Nugteren, D. H., Pabon, H. J. J., and Van Dorp, D. A. (1968). *Rec. Trav. Chim.* **87**, 461.
Bergstrom, S. (1966). *Recent Progr. Hormone Res.* **22**, 153.
Bergstrom, S. (1967a). *Proc. 2nd Nobel Symp.* p. 21. Almqvist and Wiksell, Stockholm;
Interscience, New York.
Bergstrom, S. (1967b). *Science* **157**, 382.
Bergstrom, S., and Carlson, L. A. (1965). *Acta Physiol. Scand.* **64**, 479.
Bergstrom, S., and Samuelsson, B. (1965). *Ann. Rev. Biochem.* **34**, 101.
Bergstrom, S., Carlson, L. A., and Oro, L. (1964a). *Acta Physiol. Scand.* **60**, 170.
Bergstrom, S., Danielsson, H., and Samuelsson, B. (1964b). *Biochim. Biophys. Acta* **90**,
207.
Bergstrom, S., Carlson, L. A., and Weeks, J. R. (1968). *Pharmacol. Rev.* **20**, 1.
Bennett, W. A. (1967). *J. Chem. Educ.* **44**, 17.
Bieri, J. B., Mason, K. E., and Prival, E. L. (1969). *J. Nutr.* **97**, 163.
Biran, L. A., Bartley, W., Carter, C. W., and Renshaw, A. (1964). *Biochem. J.* **93**, 492.
Biran, L. A., Bartley, W., Carter, C. W., and Renshaw, A. (1965). *Biochem. J.* **94**, 247.
Blank, M. L., and Privett, O. S. (1963). *J. Lipid Res.* **4**, 470.
Bloch, K. (1969). *Accounts Chem. Res.* **2**, 193.

- Blomstrand, R., Dhopeshwarkar, G. A., and Gustafsson, B. E. (1963). *J. Atheroscler. Res.* 3, 274.
- Bottino, N. (1967). *Lipids* 2, 155.
- Boyd, F. M., and Edwards, H. M. (1966). *Proc. Soc. Exp. Biol. Med.* 122, 218.
- Boyd, G. S. (1962). *Fed. Proc.* 21, 86.
- Bragdon, J. H., and Karmen, A. (1960). *J. Lipid Res.* 1, 167.
- Brenner, R. R., and Jose, P. (1965). *J. Nutr.* 85, 196.
- Brenner, R. R., and Nervi, A. M. (1965). *J. Lipid Res.* 6, 363.
- Brenner, R. R., and Peluffo, R. O. (1966a). *Biochim. Biophys. Acta* 137, 184.
- Brenner, R. R., and Peluffo, R. O. (1966b). *J. Biol. Chem.* 241, 5213.
- Brenner, R. R., and Peluffo, R. O. (1969). *Biochim. Biophys. Acta* 176, 471.
- Brenner, R. R., Mercuri, O., and DeTomas, M. E. (1962). *J. Nutr.* 77, 203.
- Brenner, R. R., Vazza, D. B., and DeTomas, M. E. (1963). *J. Lipid Res.* 4, 341.
- Brenner, R. R., Peluffo, R. O., Nervi, A. M., and De Tomas, M. E. (1969). *Biochim. Biophys. Acta* 176, 420.
- Burr, G. O., and Burr, M. M. (1929). *J. Biol. Chem.* 82, 345.
- Burr, G. O., and Burr, M. M. (1930). *J. Biol. Chem.* 86, 587.
- Carroll, K. K. (1965). *J. Amer. Oil Chem. Soc.* 42, 516.
- Casal, J. J., and Holman, R. T. (1965). *J. Amer. Oil Chem. Soc.* 42, 1134.
- Caster, W. O., and Ahn, P. (1963). *Science* 139, 1213.
- Caster, W. O., Ahn, P., Hill, E. G., Mohrhauer, H., and Holman, R. T. (1962). *J. Nutr.* 78, 147.
- Caster, W. O., Mohrhauer, H., and Holman, R. T. (1966). *J. Nutr.* 89, 217.
- Catala, A., and Brenner, R. R. (1967). *Lipids* 2, 114.
- Century, B., and Horwitt, M. K. (1963). *J. Nutr.* 80, 145.
- Century, B., and Horwitt, M. K. (1967). *Fed. Proc.* 26, 616.
- Century, B., and Horwitt, M. K. (1968). *J. Nutr.* 95, 509.
- Christiansen, K., Marcel, Y., Gan, M. V., Mohrhauer, H., and Holman, R. T. (1968). *J. Biol. Chem.* 243, 2969.
- Christiansen, K., Gan, M. V., and Holman, R. T. (1969). *Biochim. Biophys. Acta* 187, 19.
- Christie, W. W., and Holman, R. T. (1966). *Lipids* 1, 176.
- Christophersen, B. O. (1969). *Biochim. Biophys. Acta* 176, 463.
- Chung, A. E. (1966). *Biochim. Biophys. Acta* 116, 205.
- Coats, J. (1965). *J. Lipid Res.* 6, 494.
- Collins, F. D. (1960). *Nature* 186, 366.
- Collins, F. D. (1962). *Biochem. Biophys. Res. Commun.* 9, 289.
- Collins, F. D. (1963). *Biochem. J.* 88, 319.
- Collins, F. D. (1966). *Biochem. J.* 99, 117.
- Coots, R. H. (1964). *J. Lipid Res.* 5, 468; 5, 473.
- Decker, W. J., and Mertz, W. (1967). *J. Nutr.* 91, 324.
- DeGier, J., and Van Deenen, L. L. M. (1961). *Biochim. Biophys. Acta* 49, 286.
- De Pury, G. G., and Collins, F. D. (1963). *Nature* 198, 788.
- De Pury, G. G., and Collins, F. D. (1965). *Biochim. Biophys. Acta* 106, 213.
- De Pury, G. G., and Collins, F. D. (1966). *Chem. Phys. Lipids* 1, 1, 20.
- Dhopeshwarkar, G. A., and Mead, J. F. (1962). *J. Lipid Res.* 3, 238.
- Donaldson, W. E. (1966). *Biochem. Biophys. Res. Commun.* 24, 443.
- Du, Julie T., and Johnson, R. M., personal communication.
- Elson, C. E., Dugan, L. R., Bratzler, L. J., and Pearson, A. M. (1966). *Lipids* 1, 322.
- Enser, M., and Bartley, W. (1962). *Biochem. J.* 85, 607.
- Farquhar, J. W. (1965). *J. Amer. Oil Chem. Soc.* 42, 615.

- Ferreira, S. H., and Vane, J. R. (1967). *Nature* **216**, 873.
- Fitch, C. D., Denning, J. S., Witting, L. A., and Horwitt, M. K. (1961). *J. Nutr.* **74**, 409.
- Fulco, A. J., and Mead, J. F. (1960). *J. Biol. Chem.* **235**, 3379.
- Funch, J. P., Aaes Jorgensen, E., and Dam, H. (1957). *J. Nutr.* **11**, 426.
- Futterman, S., Rollins, M. N., and Vacano, E. (1968). *Biochim. Biophys. Acta* **164**, 433.
- Gambal, D., and Quackenbush, F. W. (1968). *Proc. Soc. Exp. Biol. Med.* **127**, 1137.
- Gassman, P. G. (1962). *Chem. Ind. (London)* p. 740.
- Gellhorn, A., and Benjamin, W. (1964). *Biochim. Biophys. Acta* **84**, 167.
- Gerschenson, L. E., Harary, I., and Mead, J. F. (1967). *Biochim. Biophys. Acta* **131**, 50.
- Glende, E. A., and Cornatzer, W. E. (1965). *J. Nutr.* **86**, 178.
- Glenn, J. L., and Dam, H. (1965). *J. Nutr.* **86**, 143.
- Glomset, J. A. (1968). *J. Lipid Res.* **9**, 155.
- Gottenbos, J. J., Beerthuis, R. K., and Van Dorp, D. A. (1967). *Proc. 2nd Nobel Symp., Stockholm* p. 57, Almqvist and Wiksell, Stockholm; Interscience, New York.
- Green, D. E., and Tzagoloff, A. (1966). *J. Lipid Res.* **7**, 587.
- Greenberg, L. D., and Moon, H. D. (1961). *Arch. Biochem. Biophys.* **94**, 405.
- Guarnieri, M., and Johnson, R. M. Unpublished results.
- Gunstone, F. D. (1966). *Chem. Ind. (London)* p. 1551.
- Hamberg, M., and Samuelsson, B. (1967a). *J. Biol. Chem.* **242**, 5329.
- Hamberg, M., and Samuelsson, B. (1967b). *J. Biol. Chem.* **242**, 5344.
- Hands, A. R., Sutherland, N. S., and Bartley, W. (1965). *Biochem. J.* **94**, 279.
- Hayashida, T., and Portman, O. W. (1960a). *Arch. Biochem. Biophys.* **91**, 206.
- Hayashida, T., and Portman, O. W. (1960b). *Proc. Soc. Exp. Biol. Med.* **103**, 656.
- Hill, E. E., Husbands, D. R., and Lands, W. E. M. (1968). *J. Biol. Chem.* **243**, 4440.
- Hill, E. G. (1966). *J. Nutr.* **89**, 465.
- Hill, J. G., Kuksis, A., and Beveridge, J. M. R. (1964). *J. Amer. Oil Chem. Soc.* **41**, 393.
- Hill, J. G., Kuksis, A., and Beveridge, J. M. R. (1965). *J. Amer. Oil Chem. Soc.* **42**, 137.
- Hochstein, P., and Ernst, L. (1963). *Biochem. Biophys. Res. Commun.* **12**, 388.
- Holman, R. T. (1960). *J. Nutr.* **70**, 405.
- Holman, R. T. (1964). *Fed. Proc.* **23**, 1062.
- Holman, R. T. (ed.) (1966). *Progr. Chem. Fats Other Lipids* **9**, Part 1.
- Holman, R. T., Egwim, P. O., and Christie, W. W. (1969). *J. Biol. Chem.* **244**, 1149.
- Holton, R. W., Blacker, H. H., and Onore, M. (1964). *Phytochemistry* **3**, 595.
- Hopkins, D. T., Witter, R. L., and Nesheim, M. C. (1963). *Proc. Soc. Exp. Biol. Med.* **114**, 82.
- Horner, A. A., and Morton, R. A. (1961). *Biochem. J.* **79**, 636.
- Horton, E. W. (1965). *Experientia* **21**, 113.
- Horton, E. W. (1969). *Physiol. Rev.* **49**, 122.
- Hyun, J., Kotheri, H., Herm, E., Mortenson, J., Treadwell, C. R., and Vahouny, G. (1969). *J. Biol. Chem.* **244**, 1937.
- Ito, T., and Johnson, R. M. (1964). *J. Biol. Chem.* **239**, 3201.
- Ito, T., and Johnson, R. M. (1968). *J. Nutr.* **96**, 215.
- Jacobs, M. H., Glassman, N., and Parpart, A. K. (1950). *J. Exp. Zool.* **113**, 277.
- Jenkins, K. J., and Ewan, L. M. (1967). *Can. J. Biochem.* **45**, 1873.
- Jensen, B., Nakamura, M., and Privett, O. S. (1968). *J. Nutr.* **95**, 406.
- Jensen, L. S. (1968). *Fed. Proc.* **27**, 914.
- Jensen, L. S., and Schutz, J. V. (1963). *Poultry Sci.* **42**, 921.
- Johnson, R. M. (1963a). *Exp. Cell Res.* **32**, 118.
- Johnson, R. M. (1963b). *J. Nutr.* **81**, 411.
- Johnson, R. M., and Ito, T. (1965). *J. Lipid Res.* **6**, 75.

- Johnson, R. M., Bouchard, P., Tinoco, J., and Lyman, R. L. (1967). *Biochem. J.* **105**, 343.
- Jones, P. D., Holloway, P. W., Peluffo, R. O., and Wakil, S. J. (1969). *J. Biol. Chem.* **244**, 744.
- Jurtschuck, P., Jr., Sekuzu, I., and Green, D. E. (1961). *Biochem. Biophys. Res. Commun.* **6**, 75.
- Kanoh, H. (1969). *Biochim. Biophys. Acta* **176**, 756.
- Kaufmann, H. P., and Mankel, G. (1964). *Fette, Seifen, Anstrichm.* **66**, 6.
- Klein, P. D., and Johnson, R. M. (1954). *J. Biol. Chem.* **211**, 103.
- Klenk, E. (1965a). *Advan. Lipid Res.* **3**, 2.
- Klenk, E. (1965b). *J. Amer. Oil Chem. Soc.* **42**, 580.
- Knipprath, W. G., and Mead, J. F. (1964). *J. Amer. Oil Chem. Soc.* **41**, 437.
- Knipprath, W. G., and Mead, J. F. (1966). *Biochim. Biophys. Acta* **116**, 198.
- Kogl, F., DeGier, J., Mulder, I., and Van Deenen, L. L. M. (1960). *Biochim. Biophys. Acta* **43**, 95.
- Kramar, J., and Levine, V. E. (1953). *J. Nutr.* **50**, 149.
- Kupiecki, F. P., and Weeks, J. R. (1966). *Fed. Proc.* **25**, 719.
- Lands, W. E. M. (1965). *J. Amer. Oil Chem. Soc.* **42**, 465.
- Lands, W. E. M., and Hart, P. (1965). *J. Biol. Chem.* **240**, 1905.
- Lands, W. E. M., Blank, M. L., Nutter, L. J., and Privett, O. S. (1966). *Lipids* **1**, 224.
- Leat, W. M. F. (1963). *Biochem. J.* **89**, 45.
- Lee, D. J., Roehm, J. N., Yu, T. C., and Sinnhuber, R. O. (1967). *J. Nutr.* **92**, 93.
- Lee, D. J., Chin, M., and Draper, H. H. (1964). *J. Nutr.* **84**, 401.
- LeGroff, E. (1964). *J. Org. Chem.* **29**, 2048.
- Levin, E., Johnson, R. M., and Albert, S. (1957). *J. Biol. Chem.* **228**, 15.
- Lillie, R. J., and Menge, H. (1968). *J. Nutr.* **95**, 311.
- Lindstrom, T., and Tinsley, I. J. (1966). *J. Lipid Res.* **7**, 758.
- Little, C., and O'Brien, P. J. (1968). *Biochem. J.* **106**, 419.
- Lowery, R. R., and Tinsley, I. J. (1966a). *Biochim. Biophys. Acta* **116**, 398.
- Lowery, R. R., and Tinsley, I. J. (1966b). *J. Nutr.* **88**, 26.
- Lyman, R. L., Ostwald, R., Bouchard, P., and Shannon, A. (1966). *Biochem. J.* **98**, 438.
- Lyman, R. L., Fosmire, M. A., Giotas, C., and Miljanich, P. (1968a). *J. Nutr.* **94**, 74.
- Lyman, R. L., Hopkins, S. M., Sheehan, G., and Tinoco, J. (1968b). *Biochim. Biophys. Acta* **152**, 197.
- Lyman, R. L., Giotas, C., Fosmire, M. A., and Miljanich, P. (1969a). *Can. J. Biochem.* **47**, 11.
- Lyman, R. L., Hopkins, S. M., Sheehan, G., and Tinoco, J. (1969b). *Biochim. Biophys. Acta* **176**, 86.
- Machlin, L. J. (1961). *Proc. Soc. Exp. Biol. Med.* **108**, 819.
- Machlin, L. J., and Gordon, R. S. (1961). *J. Nutr.* **75**, 157.
- MacMillan, A. L., and Sinclair, H. M. (1958). *Proc. 4th Intern. Conf. Biochem. Probl. Lipids, Oxford, 1957* p. 208. Butterworth, London.
- McMillan, G. C. (1964). *J. Nutr.* **83**, 314.
- Marcel, Y. L., Christiansen, K., and Holman, R. T. (1968). *Biochim. Biophys. Acta* **164**, 25.
- Marco, G. J., Machlin, L. J., Emery, E., and Gordon, R. S. (1961). *Arch. Biochem. Biophys.* **94**, 115.
- Marion, J. E., and Edwards, H. M. (1962). *Poultry Sci.* **41**, 1785.
- Mattson, F. H. (1960). *J. Nutr.* **71**, 366.
- May, H. E., and McCay, P. B. (1968). *J. Biol. Chem.* **243**, 2296.
- Mellin Koff, S. M., Frankland, M., Schwabe, A. D., Kellner, H. C., Greipel, M., and McNall, D. (1965). *Amer. J. Clin. Nutr.* **16**, 232.

- Menge, H. (1967). *J. Nutr.* **92**, 148.
Menge, H. (1968). *J. Nutr.* **95**, 578.
Menge, H., Calvert, C. C., and Denton, C. A. (1965a). *J. Nutr.* **87**, 365.
Menge, H., Calvert, C. C., and Denton, C. A. (1965b). *J. Nutr.* **86**, 115.
Menton, D. N. (1966). Ph. D. Dissertation, Brown University, Providence, Rhode Island.
Mercuri, O., Peluffo, R. O., and Brenner, R. R. (1967). *Lipids* **2**, 284.
Meyer, E. W. (ed.) (1966). *Ann. N. Y. Acad. Sci.* **137**, 403-1048.
Mohrhauer, H., and Holman, R. T. (1963a). *J. Lipid Res.* **4**, 151.
Mohrhauer, H., and Holman, R. T. (1963b). *J. Lipid Res.* **4**, 346.
Mohrhauer, H., and Holman, R. T. (1967). *J. Nutr.* **91**, 528.
Mohrhauer, H., Christiansen, K., Gan, M. V., Deubig, M., and Holman, R. T. (1967a). *J. Biol. Chem.* **242**, 4507.
Mohrhauer, H., Rahm, J. J., Seufert, J., and Holman, R. T. (1967b). *J. Nutr.* **91**, 521.
Moore, J. H., and Williams, D. L. (1965). *Biochim. Biophys. Acta* **98**, 137.
Moore, J. H., and Williams, D. L. (1966). *Biochim. Biophys. Acta* **116**, 181.
Moore, J. H., Williams, D. L., and Westgarth, D. R. (1965). *Biochim. Biophys. Acta* **106**, 145.
Moore, J. L., Richardson, T., and De Luca, H. F. (1967). *Lipids* **2**, 8.
Moore, J. L., Richardson, T., and De Luca, H. F. (1969). *Chem. Phys. Lipids* **3**, 39.
Morton, R. A., and Horner, A. A. (1961). *Biochem. J.* **79**, 631.
Mudd, J. R., Van Golde, L. M. G., and Van Deenen, L. L. M. (1969). *Biochim. Biophys. Acta* **176**, 547.
Munsch, N. (1965). *Compt. Rend. Acad. Sci.* **263**, 449.
Murty, N. L. (1960). *J. Nutr.* **72**, 451.
Murty, N. L., and Reiser, R. (1961). *J. Nutr.* **75**, 287.
Murty, N. L., Rakoff, H., and Reiser, R. (1962). *Biochem. Biophys. Res. Commun.* **8**, 372.
Nachbaur, J., and Vignais, P. M. (1968). *Biochem. Biophys. Res. Commun.* **33**, 315.
Naismith, D. J. (1962). *J. Nutr.* **77**, 381.
Nakagawa, M., and Uchiyama, M. (1969). *J. Biochem.* **65**, 673.
Niehaus, W. G., Jr., and Samuelsson, B. (1968). *European J. Biochem.* **6**, 126.
Nervi, A. M., Brenner, R. R., and Peluffo, R. O. (1968). *Biochim. Biophys. Acta* **152**, 539.
Nicolaides, N., and Woodall, A. N. (1965). *J. Nutr.* **87**, 431.
Nugteren, H. G. (1965). *Biochim. Biophys. Acta* **106**, 208.
O'Brien, P. J., and Frazer, A. C. (1966). *Proc. Nutr. Soc.* **25**, 9.
O'Brien, P. J., and Little, C. (1969). *Can. J. Biochem.* **47**, 493.
Okey, R., Ostwald, R., Shannon, A., and Tinoco, J. (1962). *J. Nutr.* **76**, 353.
Okey, R., Ostwald, R., Shannon, A., Miljanich, P., and Lyman, R. L. (1966). *J. Nutr.* **88**, 303.
Olivercrona, T. (1962). *Acta Physiol. Scand.* **54**, 295.
Ono, K., and Fredrickson, D. S. (1964). *J. Biol. Chem.* **239**, 2482.
Oshino, N., Imai, Y., and Sato, R. (1966). *Biochim. Biophys. Acta* **128**, 13.
Ostwald, R., and Lyman, R. L. (1968). *Lipids* **3**, 199.
Ostwald, R., Okey, R., Shannon, A., and Tinoco, J. (1962). *J. Nutr.* **76**, 341.
Ostwald, R., Bouchard, P., Miljanich, P., and Lyman, R. L. (1965). *Biochem. J.* **97**, 485.
Pace-Asciak, C., and Wolfe, L. S. (1968). *Biochim. Biophys. Acta* **152**, 784.
Packer, L., Deamer, D. W., and Heath, R. L. (1967). *Advan. Gerontol. Res.* **2**, 77.
Parros, F. C., and Finerty, J. C. (1954). *J. Nutr.* **54**, 315.
Patil, V. S., and Hansen, A. E. (1962). *J. Nutr.* **78**, 167.
Patil, V. S., and Magar, N. G. (1960). *Biochem. J.* **74**, 441.

- Pawar, S. S., and Tidwell, H. C. (1967). *Amer. J. Physiol.* **213**, 1350.
Pawar, S. S., and Tidwell, H. C. (1968). *Biochim. Biophys. Acta* **164**, 167.
Peifer, J. J. (1968). *J. Lipid Res.* **9**, 193.
Phillips, G. B. (1968). *Lipids* **3**, 385.
Pinter, K., Miller, O., and Hamilton, I. (1964). *Proc. Soc. Exp. Biol. Med.* **115**, 318.
Possmayer, F., Scherphot, G. L., Dubbelman, T. M. A. R., Van Golde, L. M. G., and Van Deenen, L. L. M. (1969). *Biochim. Biophys. Acta* **176**, 95.
Privett, O. S., and Blank, M. L. (1964). *J. Amer. Oil Chem. Soc.* **41**, 292.
Privett, O. S., Blank, M. L., and Verdino, B. (1965). *J. Nutr.* **85**, 187.
Privett, O. S., Nutter, L. J., and Lightly, F. S. (1966). *J. Nutr.* **89**, 257.
Pudelkewicz, C., and Holman, R. T. (1968). *Biochim. Biophys. Acta* **152**, 340.
Pudelkewicz, C., Seufert, J., and Holman, R. T. (1968). *J. Nutr.* **94**, 138.
Rahm, J. J., and Holman, R. T. (1964a). *J. Nutr.* **84**, 149.
Rahm, J. J., and Holman, R. T. (1964b). *J. Lipid Res.* **5**, 169.
Ramalingaswami, V., and Sinclair, H. M. (1951). *Brit. J. Nutr.* **5**, XI.
Ramwell, P. W., Shaw, J. E., Clarke, G. B., Grostic, M. F., Kaiser, D. G., and Pike, J. E. (1967). *Progr. Chem. Fats Other Lipids* **9**, 73.
Rand, P. G., and Quackenbush, F. W. (1965). *J. Nutr.* **87**, 489.
Rathbone, L. (1965). *Biochem. J.* **97**, 620.
Reiser, R., Murty, N. L., and Rakoff, H. (1962). *J. Lipid Res.* **3**, 56.
Reiser, R., Stevenson, B., Kayama, M., Choudhury, R. B. R., and Hood, D. W. (1963a). *J. Amer. Oil Chem. Soc.* **40**, 507.
Reiser, R., Williams, M. C., Sorrels, M. F., and Murty, N. L. (1963b). *Arch. Biochem. Biophys.* **102**, 276.
Reitz, R. C., Lands, W. E. M., Christie, W. W., and Holman, R. T. (1968). *J. Biol. Chem.* **243**, 2241.
Reitz, R. C., El-Sheikh, M., Lands, W. E. M., Ismail, I. A., and Gunstone, F. D. (1969). *Biochim. Biophys. Acta* **176**, 480.
Richardson, T., Tappel, A. L., and Gruger, E. H. (1961). *Arch. Biochem. Biophys.* **94**, 1.
Ross, E., and Adamson, L. (1961). *J. Nutr.* **74**, 329.
Rothblat, G., and Kritchevsky, D. (eds.) (1967). "Lipid Metabolism in Tissue Culture Cells" Wistar Inst. Press, Philadelphia, Pennsylvania.
Ruddon, R. W., and Johnson, J. M. (1967). *Life Sci.* **6**, 1245.
Salach, J. I., Turini, P., Hawber, J., Seng, R., Tisdale, H., and Singer, T. P. (1968). *Biochem. Biophys. Res. Commun.* **33**, 936.
Sand, D., Sen, N., and Schlenk, H. (1965). *J. Amer. Oil Chem. Soc.* **42**, 511.
Scanu, A. M., Van Deenen, L. L. M., and de Haas, G. H. (1969). *Biochim. Biophys. Acta* **181**, 471.
Schlenk, H., and Sand, D. M. (1967). *Biochim. Biophys. Acta* **144**, 305.
Schlenk, H., Sand, D. M., and Sen, N. (1964). *Biochim. Biophys. Acta* **84**, 361.
Schlenk, H., Gellerman, J. L., and Sand, D. M. (1967). *Biochim. Biophys. Acta* **137**, 420.
Schutz, J. V., and Jensen, L. S. (1963). *Poultry Sci.* **42**, 921.
Sekuzu, I., Jurtschuck, P., Jr., and Green, D. E. (1963). *J. Biol. Chem.* **238**, 975.
Selinger, Z., and Holman, R. T. (1965). *Biochim. Biophys. Acta* **106**, 56.
Seward, C. R., Vaughn, G., Shue, G. M., and Hove, E. L. (1966). *J. Nutr.* **90**, 245.
Sewell, R. F., and McDowell, L. J. (1966). *J. Nutr.* **89**, 64.
Sewell, R. F., and Miller, I. L. (1966). *J. Nutr.* **88**, 171.
Sgoutas, D. S. (1968). *Biochim. Biophys. Acta* **164**, 317.
Sinclair, A. J., and Collins, F. D. (1968). *Biochim. Biophys. Acta* **152**, 498.
Sinclair, H. M. (1952). *Biochem. Soc. Symp.* **9**, 80.
Sinclair, H. M. (ed.) (1958). "Essential Fatty Acids." Academic Press, New York.

- Sinclair, H. M. (1964). In "Lipid Pharmacology" (R. Paoletti, ed.), p. 237, Academic Press, New York.
- Smith, A. D., and Winkler, H. (1968). *Biochem. J.* **108**, 867.
- Smithson, J. E. (1967). *Anat. Record* **157**, 324.
- Solyom, A., Muhlbachova, E., and Puglisi, L. (1967). *Biochim. Biophys. Acta* **137**, 427.
- Spink, W. W., and Su, C. K. (1963). *Proc. Soc. Exp. Biol. Med.* **112**, 463.
- Spitzer, H. L., Norman, J. R., and Morrison, K. (1969). *Biochim. Biophys. Acta* **176**, 584.
- Sprecher, H. (1967). *Biochim. Biophys. Acta* **144**, 296.
- Sprecher, H. (1968a). *Biochim. Biophys. Acta* **152**, 519.
- Sprecher, H. (1968b). *Lipids* **3**, 14.
- Sprecher, H. W., Dutton, H. J., Gunstone, F. D., Sykes, P. T., and Holman, R. T. (1967). *Lipids* **2**, 122.
- Stancliff, R. C., Williams, M. A., Utsumi, K., and Packer, L. (1969). *Arch. Biochem. Biophys.* **131**, 629.
- Stearns, E. M., Rysavy, J. A., and Privett, O. S. (1967). *J. Nutr.* **93**, 485.
- Stein, A. A., Estilo, A., Soike, K., and Patterson, P. (1968). *Fed. Proc. Abstr.* **1471**.
- Stein, O., and Stein, Y. (1964). *Biochim. Biophys. Acta* **84**, 621.
- Stoffel, W. (1966). *Naturwissenschaften* **53**, 621.
- Stoffel, W., and Ach, K. L. (1964). *Z. Physiol. Chem.* **337**, 123.
- Stoffel, W., and Scheid, A. (1967). *Z. Physiol. Chem.* **347**, 102.
- Stoffel, W., and Schiefer, H. G. (1965). *Z. Physiol. Chem.* **341**, 84.
- Stoffel, W., and Schiefer, H. G. (1966). *Z. Physiol. Chem.* **345**, 41.
- Stoffel, W., Ditzer, R., and Caesar, H. (1964). *Z. Physiol. Chem.* **339**, 167.
- Sugano, M., and Portman, O. W. (1965). *Arch. Biochem. Biophys.* **109**, 302.
- Swell, L., and Law, M. D. (1965). *Arch. Biochem. Biophys.* **112**, 115.
- Swell, L., and Law, M. D. (1967). *Biochem. Biophys. Res. Commun.* **26**, 206.
- Swell, L., Law, M. D., Schools, P. W., and Treadwell, C. R. (1961). *J. Nutr.* **75**, 181.
- Swell, L., Law, M. D., and Treadwell, C. R. (1962). *J. Nutr.* **76**, 429.
- Swick, R. W., Rexroth, A. K., and Strange, J. L. (1968). *J. Biol. Chem.* **243**, 3581.
- Takasugi, T., and Imai, Y. (1966). *J. Biochem.* **60**, 191.
- Tappel, A. L. (1962). In "Lipids and Their Oxidations," (H. W. Schultz, E. A. Day, and R. O. Sinnhuber, eds.), p. 134. Avi Publ., Westport, Connecticut.
- Tappel, A. L., Shibko, S., Stein, M., and Suez, J. P. (1965). *J. Food Sci.* **30**, 498.
- Thomasson, H. J. (1962). *Nature* **194**, 973.
- Tidwell, H. C., McPherson, J. C., and Gifford, P. (1965). *J. Amer. Oil Chem. Soc.* **42**, 483.
- Tinoco, J., Babcock, R., McIntosh, D. J., and Lyman, R. L. (1968). *Biochim. Biophys. Acta* **164**, 129.
- Tinsley, I. J. (1964). *J. Food Sci.* **29**, 130.
- Tischer, K., and Glenn, J. L. (1965). *Biochim. Biophys. Acta* **98**, 502.
- Tove, S. B., and Smith, F. H. (1960). *J. Nutr.* **71**, 264.
- Trojan, L. E. (1966). Ph. D. Dissertation. The Ohio State University, Columbus, Ohio.
- Trojan, L. E., and Johnson, R. M. (1968). *J. Nutr.* **94**, 369.
- Tulpule, P. G., and Patwardhan, V. N. (1952). *Arch. Biochem. Biophys.* **39**, 450.
- Tulpule, P. G., and Williams, J. N., Jr. (1955). *J. Biol. Chem.* **217**, 229.
- Turpeinen, O. (1937). *Proc. Soc. Exp. Biol. Med.* **37**, 37.
- Uchiyama, M., Nakagawa, M., and Kui, S. (1967). *J. Biochem.* **62**, 1.
- Van Deenen, L. L. M. (1965). *Prog. Chem. Fats Other Lipids* **8**, Part 1.
- Van Den Bosch, H., Aarsman, A. J., Slotboom, A. J., and Van Deenen, L. L. M. (1968a). *Biochim. Biophys. Acta* **164**, 215.
- Van Den Bosch, H., Van Golde, L. M. G., Slotboom, A. J., and Van Deenen, L. L. M. (1968b). *Biochim. Biophys. Acta* **152**, 694.

- Van Den Bosch, H., Slotboom, A. J., and Van Deenen, L. L. M. (1969). *Biochim. Biophys. Acta* **176**, 632.
- Van Dorp, D. A., Beertuis, R. K., Nugteren, D. H., and Vonkeman, H. (1964a). *Biochim. Biophys. Acta* **90**, 204.
- Van Dorp, D. A., Beertuis, R. K., Nugteren, D. H., and Vonkeman, H. (1964b). *Nature* **203**, 839.
- Van Golde, L. M. G., and Van Deenen, L. L. M. (1966). *Biochim. Biophys. Acta* **125**, 496.
- Van Golde, L. M. G., Pietersen, W. A., and Van Deenen, L. L. M. (1968). *Biochim. Biophys. Acta* **152**, 84.
- Verdino, B., Blank, M. L., Privett, O. S., and Lundberg, W. O. (1964). *J. Nutr.* **83**, 234.
- von Euler, U.S., and Eliasson, R. (1967). "Prostaglandins," p. 33. Academic Press, New York.
- Waite, M., and Van Golde, L. M. G. (1968). *Lipids* **3**, 449.
- Waite, M., Scherphof, G. L., Boshouwers, F. M. G., and Van Deenen, L. L. M. (1969). *J. Lipid Res.* **10**, 411.
- Walker, B. L. (1966). *Arch. Biochem. Biophys.* **114**, 465.
- Walker, B. L. (1967a). *Lipids* **2**, 497.
- Walker, B. L. (1967b). *J. Nutr.* **92**, 23.
- Walker, B. L. (1968). *J. Nutr.* **94**, 469.
- Walker, B. L., and Kummerow, K. A. (1963). *J. Nutr.* **81**, 75.
- Weeks, J. R. (1966). Personal communication.
- Wells, M. A., and Hanahan, D. J. (1969). *Biochemistry* **8**, 414.
- Willebrands, A. F., and Van Der Veen, K. J. (1966). *Biochim. Biophys. Acta* **116**, 583.
- Williams, M. A., Chu, L. C., McIntosh, D. J., and Hincenberg, I. (1968). *J. Nutr.* **94**, 377.
- Wills, E. D. (1969). *Biochem. J.* **113**, 315, 325, 333.
- Wilson, H. W., and Le Duc, E. (1963). *J. Cell Biol.* **16**, 281.
- Witting, L. A., Harvey, C. C., Century, B., and Horwitt, M. K. (1961). *J. Lipid Res.* **2**, 412.
- Yacowitz, H., Fleischman, A. I., Amsden, R. T., and Bierenbaum, M. L. (1967). *J. Nutr.* **92**, 389.
- Yamamoto, A., Isozaki, M., Hirayama, K., and Sakai, Y. (1965). *J. Lipid Res.* **6**, 295.
- Yu, B. P., Kummerow, F. A., and Nishida, T. (1966). *J. Nutr.* **89**, 435.
- Yu, B. P., DeMartinis, F. D., and Masoro, E. J. (1968). *J. Lipid Res.* **9**, 492.
- Zahler, W. L., and Cleland, W. W. (1969). *Biochim. Biophys. Acta* **176**, 699.
- Zmachinski, H., Waltking, A., and Miller, J. D. (1966). *J. Amer. Oil Chem. Soc.* **43**, 525.