The Physiology and Biochemistry of the Essential Fatty Acids

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I. Introduction

The first proof that fats comprise a necessary component in the diet was adduced in 1926 by Evans and Burr. Shortly thereafter, McAmis *et al.* (1929) also reported that rats grew better on fat-containing diets than on regimens deficient in this foodstuff; in fact, the latter workers reported deficiency symptoms in rats which received the fat-free diets. It remained for Burr and Burr (1929) to demonstrate in a clear-cut manner that the beneficial effect of fat on growth was due to the fact that it prevented a dietary disease. This deficiency condition was not to be ascribed to the lack of vitamins A and D, but rather to the absence of components present in the saponifiable fraction. The latter active compounds were shown to be polyunsaturated fatty acids, which are usually referred to as essential fatty acids (EFA). The fat-deficiency symptoms produced by a fat-free diet were found to disappear in a dramatic fashion when certain unsaturated fats, or linoleic, linolenic, or arachidonic acids, were fed (Burr and Burr, 1930). Earlier reviews of the nutritional significance of the EFA included that of Burr (1942), and of

Sherman (1950), and two by the present author (Deuel and Greenberg, 1950; Deuel, 1954a).

II. Chemical Relations of Polyunsaturated Fatty Acids

1. Polyunsaturated Acids Having Biological Importance

The polyunsaturated fatty acids, which were first recognized by Burr and Burr (1930) as possessing biopotency in curing or preventing the fat-deficiency disease produced by the rigid exclusion of fat from the diet, were *linoleic acid*, 9,12-octadecadienoic acid, *linolenic acid*, 9,12,15-octadecatrienoic acid, and *arachidonic acid*, 5,8,11,14-eicosatetraenoic acid. Hume *et al.* (1938) reported that a docosahexaenoic acid of unknown structure possesses similar biological activity. Moreover, methyl linoleate and linoleyl alcohol, 9,12-octadecadienol, were found by Turpeinen (1937, 1938) to possess biopotency, presumably because they are converted to arachidonic acid in the body in the course of their intermediary metabolism.

On the other hand, several polyunsaturated acids have been found to be biologically inactive in respect to the fat-deficiency syndrome. Included in this category are the following acids: linolelaidic acid (*trans*-9-*trans*-12-octadecadienoic acid), as reported by Burr (1942), 9,11-linoleic acid (9,11-octadecadienoic acid), as recorded both by Burr (1942) and by Thomasson (1953), who employed a new bio-assay procedure. 10,12-Octadecadienoic acid was likewise reported by the latter investigator to be biologically inactive. Other dienoic acids concerning which data are available include 2,6-phytadienoic acid (Karrer and Koenig, 1943), which is inactive; 10,13-nonadecadienoic acid, reported as inactive by Karrer and Koenig (1943), and as possessing 9% of the activity of linoleic acid by Thomasson (1953); and 11,14-eicosadienoic acid, also listed as practically inactive by Karrer and Koenig (1943), but to which a value of 43% of that of linoleate was attributed by Thomasson (1953). Sorbic acid (2,4-hexadienoic acid) has likewise been reported to be biologically inactive (Deuel, Alfin-Slater, Weil, and Smyth, 1954a).

Thomasson (1953) noted that ordinary linolenic acid (9,12,15-octadecatrienoic acid), formerly considered as possessing biopotency equal to that of linoleic acid, under certain conditions, is actually practically inactive (9%) when tested by the new bio-assay method of the author; on the other hand, γ-linolenic acid (6,9,12-octadecatrienoic acid) is considered to possess 100% biopotency as compared with linoleate. The conjugated trienoic acid, α-elaeostearic acid (9,11,13-octadecatrienoic acid), was reported by Burr, Burr, and Miller (1932) to be ineffective in counteracting the fat-deficiency syndrome; confirmatory negative results have

been recorded by Deuel *et al.* (1954a, 1955). Tange (1932) likewise noted that 4,8,12,15,19-docosapentaenoic acid gives a negative biological response.

Thomasson (1953) is of the opinion that the presence of double bonds at the 6:7 and the 9:10 positions (counting from the terminal CH_3— group (or ω-carbon)) is essential for the biological activity of the unsaturated fatty acids. When the number of double bonds is extended on the CH_3— side of these afore-mentioned positions, the bioactivity decreases, whereas dehydrogenation on the carboxyl side does not weaken the EFA activity but may actually augment it.

2. Methods of Preparation of Polyunsaturated Acids

a. Introduction. The preparation of pure polyunsaturated fatty acids still poses a very difficult problem. This is especially the situation in the case of arachidonic acid; variations in biological response to this acid have been attributed to differences in the purity of the samples assayed.

If the source of the polyunsaturated acid is a tissue rather than a pure fat or oil, the total lipid must first be extracted by some method of wet extraction. For example, Holman and Greenberg (1953) employ ethanol, or ethanol-ether, as a solvent, or separate the acid after digestion of the tissue with 30% potassium hydroxide. It is important to protect the sample as much as possible during all stages of preparation, and for this reason the reactions are generally carried out as far as is practicable under nitrogen. After isolation of the lipid mixture, the material is saponified (if this step has not been previously carried out), and the acid is freed, washed, and dried.

The saturated fatty acids may be removed from the mixture by the lead soap ether or the lead soap alcohol method; these methods depend upon the relative insolubility of the lead soaps of the saturated acids in organic solvents.

b. Low-Temperature Crystallization. The lead soap method for the separation of the saturated acids has been largely replaced by several newer procedures. One of these methods, which involves low-temperature crystallization from organic solvents, was developed in the laboratories of J. B. Brown 20 years ago. The features of the procedure were reviewed by Brown (1941). The low-temperature fractionation not only facilitates separation of saturated from unsaturated acids but also renders possible the preparation of samples of oleic and linoleic acid of high purity. However, highly purified linolenic acid cannot be prepared by this method.

c. The Urea-Adduct Method. The use of the urea inclusion compounds for the preparation of pure saturated and unsaturated compounds has

proved a simple and highly effective method for the preparation of a number of saturated and unsaturated acids. According to H. Schlenk (1954), who recently published a comprehensive review on this subject, the compounds which form urea adducts must necessarily have more than four to six carbon atoms, and the carbons must be linked to form a straight chain. The reaction of urea to form compounds with fats was discovered by Bengen (as described by W. Schlenk, Jr., 1949, 1950) in the case of milk fats. It was later noted that urea inclusion compounds were formed with straight-chain but not with branched-chain molecules (Bengen and W. Schlenk, Jr., 1949). Bengen (1940) patented these findings under the title "Method for Separation of Aliphatic Oxygen-Containing Compounds and of Straight-Chain Hydrocarbons of at Least 6 Carbon Atoms from Mixtures Containing Them."

H. Schlenk and Holman (1950) described the separation of urea inclusion compounds, of increasing degrees of unsaturation, by the fractional precipitation of a mixture of fatty acids with methanol solutions of urea. Reiser (1950b), in studies of fat absorption, employed a similar procedure for the preparation of conjugated linoleic acid. Swern and Parker (1953) prepared linoleic acid of 85% to 95% purity from corn oil, in a 72% to 50% yield, and 87% to 89% pure linolenic acid, in a yield of 71% to 55%, from perilla oil. Achaya *et al.* (1954b) obtained linoleic acid with an iodine number of 172 to 175 (theory 181.4) in a single operation from safflowerseed fatty acids.

Abu-Nasr, Potts, and Holman (1954) employed the urea inclusion technique in an effort to prepare fatty acids having four, five, or six double bonds from marine fish oil; although they were unsuccessful, they did obtain fractions with iodine numbers as high as 356.

d. Preparation from Bromo-Derivatives. One of the most widely used procedures for the preparation of certain polyunsaturated acids is by decomposition of the corresponding bromo-derivatives. Under certain conditions, when the double bonds are saturated by bromination, the halogen compounds possess solubility differences in organic solvents which permit their isolation (Markley, 1947).

However, at least two drawbacks have been ascribed to the use of this procedure for the preparation of the EFA. Thus, in the first place, Frankel and Brown (1943) showed that the unsaturated acids formed after debromination consist of mixtures of *cis-* and *trans*-isomers. Since the geometrical isomerization is an important factor in establishing whether or not a product may possess biological activity, this criticism merits most serious consideration. A second difficulty associated with the use of the debromination method in the preparation of polyunsaturated acids is its limited application. Thus, the procedure cannot be applied to acids

which have four or more double bonds, since these bromo-compounds are too insoluble to allow their separation in a satisfactory manner from a solvent; moreover, such polybromo-derivatives prove to be difficult to debrominate.

e. Chromatographic and Related Methods. The only methods which permit certain highly unsaturated acids to be obtained in a high degree of purity in their natural form are the newer procedures of chromatography. Elution analysis, frontal analysis, displacement analysis, partition chromatography, and paper partition chromatography have all been employed.

Partition chromatography has been used over the past decade for the separation and analysis of volatile fatty acids, in which chloroform + 1% butanol, along with silica gel, was the resolving mixture (Smith, 1945; Ramsey and Patterson, 1945; and Elsden, 1946). Moyle *et al.* (1948) were able to effect the separation of a somewhat wider range of saturated fatty acids (C_2 to C_8) by the additional precaution of buffering the silica gel tube. Peterson and Johnson (1948) reported that the even-chain acids from C_2 to C_{10}, and also formic acid, could be separated by employing benzene-aqueous sulfuric acid in Celite-packed tubes. This procedure is the first which has found application in the fractionation of unsaturated fatty acids (Reinbold and Dutton, 1948); oleic and linoleic acids could be effectively separated by this method to a degree not possible by fractionation. Abu-Nasr and Holman (1954) isolated docosahexaenoic acid from cod-liver oil by adsorption on charcoal in a modified Tiselius-Claesson apparatus, as described by Holman and Hagdahl (1951). Abu-Nasr and Holman (1954) also reported the preparation of eicosapentaenoic acid of 91% purity by the preliminary concentration of the saturated acid on silicic acid, followed by its adsorption on charcoal in the modified Tiselius-Claesson apparatus. When silicic acid was used as the adsorbent and a petroleum ether-chloroform mixture as the eluant, these workers succeeded in preparing almost pure ethyl docosapentaenoate.

Some of the highly purified esters of the polyunsaturated acids were prepared by the workers at the Eastern Regional Research Laboratories of the United States Department of Agriculture, by adsorption on silicic acid followed by fractionation. The products prepared included the methyl esters of natural linoleic and linolenic acids (Riemenschneider *et al.*, 1949), almost pure methyl arachidonate (Herb, Riemenschneider, and Donaldson, 1951a), methyl eicosapentaenoate and methyl docosapentaenoate (Herb, Witnauer, and Riemenschneider, 1951b). In general, the procedure involved the separation of oleic acid from the mixture by crystallization from acetone at a low temperature, the subsequent removal of the nonsaponifiable matter, the preparation of the methyl esters of the

fatty acids, followed by their adsorption on silicic acid. Elution was carried out with petroleum ether containing 0.25% dimethyl ether.

Simmons and Quackenbush (1953) employed a combined technique of chromatographic separation and elution of 2,4-dinitrobenzene-sulfenyl chloride derivatives of unsaturated acids as a new "isolation" method of analysis. The saturated acids do not react with 2,4-dinitrobenzenesulfenyl chloride, whereas the derivatives of the unsaturated acids were found to be separable into well-defined bands on $MgSO_4$ columns; they could be recovered as individual compounds in an eluate of benzene/ether (95/5), with yields above 95% in each case (oleic, linoleic, and linolenic acids). Zechmeister (1950) reviewed the subject of partition chromatography, and Holman (1953) has more recently discussed the subject of separation of fatty acids from a somewhat broader viewpoint.

3. Chemical and Physical Methods for the Analysis of Polyunsaturated Acids

The qualitative and quantitative determinations of the polyunsaturated acids have posed exceedingly refractory problems, and they have only recently been partially resolved. The former methods were tedious and of such uncertain interpretation that they were of little value. Frequently, they were resolved by the very unsatisfactory expedient of measuring changes of iodine value. It is obvious that only the simplest generalizations can be arrived at by such procedures.

a. Thiocyanogen Number. With the advent of the thiocyanogen number test for the analysis of fats evolved by Kaufmann (1926a), it became possible to determine the unsaturated fatty acids, both qualitatively and quantitatively, with considerably more facility and precision than had hitherto been the case. Thiocyanogen reacts quantitatively with monoethenoid acids, but with only one unsaturated linkage of diethenoid acids, and with only two of the three double bonds of triethenoid acids. By combining the information obtained from the iodine value and from the thiocyanogen number, Kaufmann (1925, 1926a, 1926b) was able to predict the unsaturated fatty acid composition of fatty acid mixtures. The procedure has been standardized by Lambou and Dollear (1945, 1946), and it is now an official method of the American Oil Chemists' Society (*Official and Tentative Methods*, 1946).

b. Solubility of Bromide Derivatives. The relative solubilities of the bromides of the unsaturated acids were employed by the Brown group for the estimation of polyunsaturated acids (Ault and Brown, 1934a; Shinowara and Brown, 1938; Brown and Frankel, 1938; White and Brown, 1949). However, because of the partial solubilities of the bromides, the mutual solubility effects, the development of isomers, and also the

differences in the solubilities of the isomers, this procedure is much less satisfactory for quantitative assays than are some of those recently proposed.

c. Spectrophotometric Determination. Chemical and metabolic studies on the polyunsaturated acids received a tremendous impetus as a result of the observation of Moore (1937) that methylene-interrupted double bonds in polyunsaturated fatty acids become partially conjugated upon prolonged saponification. Since conjugated double bonds absorb ultraviolet light of specific wavelengths, whereas methylene-interrupted double bonds do not possess this property, this discovery suggested a procedure which might be employed as a quantitative assay for the qualitative and quantitative determination of small amounts of polyunsaturated fats and oils.

Mitchell, Kraybill, and Zscheile, in 1943, proposed the first standardized empirical procedure for ascertaining the saturated, and mono-, di-, and tri-ethenoid acids by the use of alkali isomerization and spectrophotometric measurements, combined with the determination of the iodine values. The use of this procedure was extended to tetraenoic acids, in 1944, by Beadle and Kraybill, who reported the extinction coefficient for arachidonic acid.

The alkali-isomerization method for the determination of the polyunsaturated acids now generally in use is essentially that described by Brice and co-workers (1952), who employed new standards for extinction coefficients. These standards were prepared from "natural" acids by chromatographic procedures, rather than those used by Brice and Swain (1945) and by Brice *et al.* (1945), which included bromination-debromination techniques.

The Spectroscopy Committee of the American Oil Chemists' Society (Stillman, 1949) has developed a modification of the above method in which ethylene glycol is used for the saponification medium and in which nitrogen gas is employed to prevent oxidation. With some modifications, the same procedure has been accepted by the American Oil Chemists' Society as a tentative method.

Although the AOCS method can be carried out with 50 to 100 mg. of sample, micromethods have been devised for use when limited amounts of fat are available. Thus, Herb and Riemenschneider (1953) devised a procedure which requires only 1 to 10 mg. of fat, and two microprocedures have been devised for the determination of polyunsaturated acids in blood (O'Connell *et al.*, 1952; Wiese and Hansen, 1952).

d. "Isolation" Method of Simmons and Quackenbush. The results obtained by analysis for polyunsaturated acids, using the "isolation" method of Simmons and Quackenbush (1953), compare closely with those

determined by spectrophotometric determination following alkali isomerization. This new method involves the separation of compounds of the unsaturated fatty acids when they react with 2,4-dinitrobenzene-sulfenyl chloride; these derivatives can be separated into well-defined bands on $MgSO_4$ columns, from which they can be eluted separately. The amount of the sample employed with vegetable oils was 100 mg.

e. Résumé of Methods for the Analysis of Polyunsaturated Acids. The spectrophotometric method involving the alkali isomerization of polyunsaturated fatty acids is the most accurate and sensitive technique for the determination of these several unsaturated acids. This procedure, however, does offer some limitations. Thus, in the determination of linoleic or linolenic acids in cod-liver oil, it is practically impossible to correct for the presence of the highly unsaturated acids. The best results, with this procedure, are obtained with plant fats in which these highly unsaturated acids are not present. This method can be employed in studies of animal fat when the highly unsaturated acids are absent, or when they are present in a relatively insignificant amount as compared with the dienoic and trienoic acids. However, since the procedure is empirical, and the extinction coefficients are obtained from the literature, it is imperative that all conditions of the analysis be rigidly observed. The degree of alkali isomerization varies with the degree of alkalinity, the solvent, the temperature, and the time. When all conditions are scrupulously fulfilled, the agreement between different determinations is good. However, Deuel, Greenberg, Anisfeld, and Melnick (1951) showed that, although there is usually agreement between the EFA content determined by the alkali-isomerization method and by the growth bio-assay, this is not invariably true. In the case of a shortening prepared by nonselective hydrogenation, the bio-assay method gave a value approximately twice that obtained by the spectrophotometric procedure. It is suggested that there may be isomers of unsaturated fatty acids which are biologically active but in which conjugation cannot be effected by the alkali treatment. It would therefore appear to the authors that the absolute index of EFA content can be obtained only when the sample is tested by a bio-assay procedure.

4. Synthesis of Polyunsaturated Acids

Although the polyunsaturated acids appear superficially to be rather simple organic compounds, only the dienoic acid, linoleic acid, has been satisfactorily prepared synthetically. The synthesis of this acid was recently reported almost simultaneously by workers in three different laboratories (Walborsky *et al.*, 1951; Gensler and Thomas, 1951; and Raphael and Sondheimer, 1950). The procedures employed were essen-

tially the same and depended upon the synthesis of a long-chain acetylenic acid (9,12-octadecadiynoic acid) by the method of Ahmad, Bumpus, and Strong (1948), followed by partial hydrogenation to produce the corresponding *cis*-ethylenic acid.

A method of synthesis which should prove useful for the preparation of carboxyl-labeled linoleic acid has been recently announced by Howton and co-workers (1952). In this procedure tetrabromstearic acid was produced by bromination of natural linoleic acid. After decarboxylation of the silver salt of the tetrabrom-acid with bromine, the compound was debrominated with zinc, and the linoleic acid was regenerated with a new carboxyl group by means of the Grignard reagent with CO_2.

III. Bio-Assay of Essential Fatty Acids

1. Growth Method of Greenberg and Co-Workers

When weanling rats are fed diets complete in all essential dietary components with the exception of the EFA, retardation of the growth of the animals begins within several weeks, and the body weight reaches a plateau after 9 to 10 weeks (Deuel, Greenberg, Calbert *et al.*, 1950). From then on, the body weight may remain fairly constant over an extended period of time, or it may gradually decline, and the rats may succumb. If EFA are fed to the depleted rats on a basal fat-free diet after a constant body weight has been established, an increase in body weight promptly occurs. Within certain limits, the increase is proportional to the amount and potency of the active compound employed. Greenberg and associates (1950) reported that, when the log dose of linoleate fed was plotted against the gain-in-weight of male rats for doses of linoleate of 5, 10, 20, or 50 mg. per day, a straight-line function obtained promptly at 3 weeks, and continued for as long as 12 weeks, at which time the experiment was terminated. By comparing the gain-in-weight induced by a test substance fed at several levels with that noted when standardized doses of linoleate are used, it is possible to calculate the linoleate equivalent of the unknown.

Although the rationale of the growth method is a sound one, since it is based upon the same procedure as the one suggested by Coward (1938) for the bio-assay of vitamin A, there are several difficulties in the application of the test to the EFA. In the first place, the period of 20 weeks required for the depletion and assay periods is excessive. The diet, which contains vitamin-test casein, is expensive. Finally, the amount of the test substance which must be administered to ensure that a sufficient dose of EFA will be given may be so great that it constitutes a serious difficulty in the feeding. In the case of margarines and butters, the supplements were fed daily in 250 and 500 mg. doses (Deuel, Greenberg,

Anisfeld *et al.*, 1951). The administration of this amount of fat in the test groups was compensated for in the control linoleate tests by giving proportionate doses of hydrogenated coconut oil. Since the hydrogenated coconut oil was found to decrease the period of depletion, although the growth effect during the assay period was counteracted by linoleate, the absolute results from the assay may be open to some question (Deuel, Alfin-Slater, Wells *et al.*, 1955). It would now seem preferable to use linoleate-free, partially hydrogenated triolein as the compensating fat in such bio-assays.

2. Growth Method of Thomasson Based upon Rationing of Water

The new weight increase method proposed by Thomasson (1953) is based upon the disturbed water metabolism which occurs in EFA deficiency. As early as 1930, Burr and Burr reported that rats on fat-free diets drink more water than normal animals do. In the new bio-assay procedure, only weanling male rats are used. Animals weighing approximately 40 gm. at weaning from mothers who had received the Sherman diet were transferred to a fat-free diet. During the first two weeks on the diet, the water intake of the rats was not limited; after that the intake of water was limited to 14 ml. per rat per day for the rest of the depletion period and during the period of bio-assay. The preparatory depletion period lasted for five weeks (three weeks with water rationing); after this, some groups of animals were dosed with appropriate levels of linoleate, and other groups received the test substance five times per week during the four-week bio-assay period. The linoleate and test substance were given in doses made up to a total volume of 0.2 ml. with hydrogenated coconut oil. The determination of the quantity of essential fatty acids is based upon the slope of the gain-in-weight/log dose curve obtained, compared with that for the standard. Instead of using linoleate directly as a standard, Thomasson (1953) preferred to employ sunflowerseed oil in doses of 10 and 50 mg. One Unit of EFA (also referred to by Thomasson as vitamin F) is defined as the activity of 10 mg. of linoleic acid. Thus, one Unit corresponds almost exactly with a 1% value of EFA. Thomasson (1953) reported numerous bio-assays with this new procedure which are, in general, consistent with other available data. This procedure has the distinct advantage that the total period required is only nine weeks.

IV. Comparative Biopotencies of the Essential Fatty Acids

1. Introduction

Linoleic, linolenic, and arachidonic acids have been recognized as the chief polyunsaturated fatty acids which possess an appreciable bio-

potency in counteracting the fat-deficiency symptoms in rats, and in allowing a normal growth and metabolism in animals on a fat-free diet. Of these acids, linoleic and linolenic occur as components of many vegetable oils, whereas arachidonic acid is present exclusively in animal fats. Linoleic acid and, in some cases, linolenic acid, are likewise present in the blood and tissue fat of some animals, but it is usually considered that arachidonic acid represents the active biological form of EFA. There is excellent evidence that linoleic acid can be transformed to arachidonic acid in the animal body. It has generally been considered that the linolenate molecule is convertible into arachidonate, but at a somewhat lower order of efficiency than is the case with the linoleate molecule.

2. Proof of the Inability of Animals to Effect a Biosynthesis of the Polyunsaturated Acids

The necessity of polyunsaturated fatty acids as dietary components is to be ascribed to the fact that they cannot be synthesized by the animal *de novo*. If they are absent from the diet, tissues in which they play an integral role and enzyme systems in which they may participate will become depleted. They will then function less effectively, or they may disappear entirely.

It has long been recognized that the animal can synthesize saturated fatty acids from carbohydrate; it is now known that two-carbon intermediates, which originate from carbohydrates, participate in this biosynthesis, together with Coenzyme A. Moreover, it has been convincingly demonstrated by Schoenheimer and Rittenberg (1936), by the use of tagged stearate molecules, that the mouse is able to bring about the desaturation of stearic acid into oleic acid. Palmitoleic acid has likewise been shown to originate from palmitic acid (Stetten and Schoenheimer, 1940), while a similar desaturation of myristic acid was shown to occur, resulting in the formation of the monoethenoid acid, myristoleic acid (Anker, 1952).

In sharp contrast to the ease of synthesis of the monoethenoid acids from the corresponding saturated fatty acids, Bernhard and Schoenheimer (1940) demonstrated in an unequivocal manner that the rat is unable to synthesize either the diethenoid acid, linoleic, or the triethenoid acid, linolenic, from carbohydrate, although large amounts of stearic and palmitic acids were shown to originate concomitantly from this foodstuff. Thus, saturated and monoethenoid acids, which have most important functions in the animal, are considered to be nonessential from a dietary standpoint, since they can be synthesized even on nonfat diets. On the other hand, linoleic, linolenic, and arachidonic acids are regarded as essen-

tial, inasmuch as they cannot be synthesized in the tissues from nonfat sources. These acids must be present in the diet, as such, or a deficiency may occur in the tissues.

3. The Question of the Possible Biopotency of Oleic Acid and Other Monoethenoid Acids

Burr and Burr (1929) were originally of the opinion that oleic acid might possess some activity in counteracting the fat-deficiency syndrome. However, these workers later reported that the monoethenoid acid was completely devoid of bioactivity (Burr and Burr, 1930; Burr *et al.*, 1932). Evans and Lepkovsky (1932b) also reported the negative effect of oleic acid in preventing fat deficiency. Moreover, it has recently been observed that triolein affords no protection against X-irradiation injury (Cheng, 1954), and that the administration of methyl oleate is ineffective in counteracting the abnormal cholesterol accumulation in the liver which occurs in fat deficiency (Alfin-Slater *et al.*, 1954a,b). These results leave no doubt that oleic acid is entirely ineffective in preventing or curing the deficiency brought about by the exclusion of EFA from the diet.

In addition to the negative response to oleic acid, Thomasson (1953) reported that the following closely related monoethenoid acids likewise lack any biopotency: 11-octadecenoic acid; 12-octadecenoic acid; 13-docosenoic acid (erucic); and 12-hydroxy-9-octadecenoic acid (ricinoleic). Turpeinen (1938) also noted that 12-octadecenoic acid, erucic, and ricinoleic acids, as well as chaulmoogric acid, are without biological activity. Elaidic acid, the *trans*-isomer of oleic acid, has likewise been reported as without curative effect in fat deficiency. It is thus obvious that none of the monoethenoid acids affords protection from fat deficiency.

4. The Biopotency of Linolenic Acid vs. Linoleic Acid

Burr and co-workers (1932) stated that linoleic and linolenic acids possess about equal potency in protecting against the fat-deficiency syndrome. However, a number of workers have disagreed with these original observations. For example, Martin (1939) obtained negative results with the triethenoid acid, and Hume and collaborators (1938) stated that linolenic acid has only one-sixth of the biopotency of linoleic acid. On the other hand, Tange (1932) noted that these acids have the same biological activity. More recently, Greenberg *et al.* (1950) reported that linolenic acid, when given alone, was practically without activity to restore growth in fat-deficient rats. However, when the triethenoid acid was administered together with linoleic acid, the effect of linolenic acid appeared to be equivalent to that of linoleic acid, and the effects of the two acids were additive.

Thomasson (1953), using his new bio-assay technique for the EFA, reported a new finding which may possibly explain the divergent results of the several laboratories in regard to the biological activity of ordinary linolenic acid. Thus, it was observed that ordinary linolenic acid (9,12,15-octadecatrienoic acid) possesses practically no EFA activity (only 9%). On the other hand, isolinolenic acid (the so-called γ-linolenic acid, which is chemically 6,9,12-octadecatrienoic acid) has 100% of the biopotency of linoleic acid. Although γ-linolenic acid is known to occur in the seeds of the evening primrose (*Oenothera biennis*), its distribution is limited. It is uncertain whether or not ordinary linolenate may be activated by being transformed to the γ-isomer. More studies are needed to clear up the relationship of the several linolenic acids as possible components of the EFA. Finally, Burr *et al.* (1932) and more recently Deuel, Alfin-Slater, Weil, and Smyth (1954a) have proved that another natural trienoic acid, *i.e.*, α-elaeostearic acid (9,11,13-octadecatrienoic acid), which is present in tung oil, is completely devoid of biological potency.

5. The Biopotency of Arachidonic Acid vs. Linoleic Acid

In the original report of Burr and collaborators (1932), methyl arachidonate was listed as being somewhat inferior to linoleate as a source of EFA. These workers were unable to rationalize this observation, since the arachidonate content is known to be high in liver fat and in lard, both of which possess a high curative action against the fat-deficiency syndrome. Later workers (Turpeinen, 1938; Hume *et al.*, 1940; Greenberg, Calbert, Deuel, and Brown, 1951a), however, have assigned to arachidonic acid potencies two or three times that of linoleic acid. On the other hand, Thomasson (1953) has recently ascribed a value of only 131% to the tetraenoic acid, as compared with that of linoleate.

The reasons for the divergence in bioactivity reported by the different groups of workers are not entirely clear. In the original tests of Burr *et al.* (1932), the low value for arachidonate may have been due to the fact that "some of the purified arachidonic acid which we have fed has been altered in the process of preparation." Even so, Burr *et al.* (1940) were unable to confirm the high potency of arachidonate reported by Turpeinen (1938) when they used the same sample of acid. However, in spite of this single negative finding, the consensus is that arachidonic acid is the most active member of the EFA.

6. The Biological Activity of Related Compounds

In addition to the three main EFA, linoleyl alcohol (Turpeinen, 1938), docosahexaenoic acid, and two hexahydroxystearic acids (linusic and

isolinusic) (Hume *et al.*, 1938) have been reported to exhibit low degrees of activity in protecting against fat-deficiency symptoms. However, Thomasson (1953) recently observed that linusic acid possessed no bioactivity, and that the potency of isolinusic acid was only slight. In addition, Thomasson (1953) recorded a biopotency of 43% for 11,14-eicosadienoic acid and one of 9% for 10,13-nonadecadienoic acid. According to this author, the presence of double bonds on the 6:7 and 9:10 positions (counting from the terminal CH_3 group, or ω-carbon) is required for EFA activity. On the one hand, an increase in the number of double bonds on the CH_3-side appears to decrease the biopotency, whereas, on the other hand, an increased unsaturation on the carboxyl side not only does not decrease the biological activity but may even potentiate it.

V. Factors Affecting the Requirement for Essential Fatty Acids

1. The Effect of Species

Although the discovery of the requirement for EFA as a dietary constituent was first made on the rat, and considerable information has been obtained by the study of this species, the necessity of these acids has been found to be very widespread. Fraenkel and Blewett (1946) demonstrated that the EFA are required by certain insects, such as the Mediterranean flour moth (*Ephestia kühniella*), the tobacco moth (*E. eutella*), the fig moth (*E. cautella*), and the Indian-meal moth (*Plodia interpunctella* (Lep.)).

Mice develop a deficiency similar to that of rats on regimens devoid of EFA (White *et al.*, 1943); in fact, this species has recently been used by Decker *et al.* (1950) for a comprehensive study of EFA. Although Russell and co-workers (1940) were unable to provoke a fat deficiency in chickens, Reiser (1950a) was later successful in producing the deficiency in this species. In the latter tests, sucrose was used in the diet in place of the ground yellow corn employed by Russell *et al.* (1940). It was suggested that, in the earlier tests, there was a sufficient supply of EFA, chemically combined with the starch particles and not removed by the usual methods of extraction, to prevent EFA deficiency.

The hog represents a species relatively refractory to EFA deficiency. The failure to induce a fat deficiency in this species may be related to the high content of EFA frequently stored in its tissues. Thus, Ellis and Isbell (1926a,b) noted that lard from hogs on a high-soybean diet sometimes had a linoleate content as high as 31.9%. When the animals were subjected to a fat-free diet, it was found that the linoleate content decreased to as little as 1.3% (Ellis and Zeller, 1930). It would appear to

the reviewers that fat deficiency has not been observed in the hog because the tissue linoleate is sufficient to protect the animal on a fat-free diet for an extended period. On the other hand, Witz and Beeson (1951) have described a fatty acid deficiency in the pig.

Definite evidence of the role of the EFA in calves has only recently become available. In fact, Gullickson *et al.* (1941) were unable to demonstrate that essential fatty acids were required by calves; when vegetable oils were homogenized into skimmed milk, the resulting product could not be tolerated by the calves. Most of the animals did poorly, and a number of them died, in contradistinction to the relatively satisfactory results when whole milk was used. Although Gullickson and co-workers (1953) indicated more recently that the replacement of butterfat with vegetable oils caused some symptoms characteristic of vitamin E deficiency, they have likewise reported the normal development of a calf maintained for over 45 days on a skim-milk diet into which partially hydrogenated vegetable oil was homogenized. It has also been impossible to produce fat deficiency in cows (Gibson and Huffman, 1939; Maynard *et al.*, 1939), although a decrease in the iodine value of the blood lipids was shown to obtain when the animals were continued on a fat-free diet over a prolonged period. The recent studies of Lambert *et al.* (1954) have left no doubt that the calf is susceptible to EFA deficiency. Thus, these workers observed a marked retardation of growth of 50% of the calves on a fat-deficient regimen; scaly dandruff was also noted, together with an excessive loss of hair on the back, shoulders, and tail. These characteristic symptoms of fat deficiency were promptly alleviated when sources of the EFA were administered.

The dog represents a species which is readily susceptible to the fat-deficiency syndrome. Because of the convenient size of this species, which will enable a clinical study to be made on one animal over a period of time, considerable work has been reported on EFA deficiency in these animals. Hansen and Wiese (1943) first demonstrated that the symptoms of fat deficiency in the dog are similar to those produced by fat-free diets in other species. It has been suggested that dietary fat may supply a factor necessary for the maturation of epithelial, sebaceous, and sudoriparous cells. The fat-deficiency symptoms in the dog were found to respond quickly to a diet containing fresh lard to the extent of 29% of the total calories (Hansen and Wiese, 1943, 1951).

There is less absolute proof that the EFA are required by man than is the case for other species. Gröer (1919) and Hansen (1937) reported that a characteristic eczema develops in infants on a fat-low diet which rapidly responds when fat is added to the restricted regimen. Although neither Taub and Zakon (1935) nor Ginsberg *et al.* (1937) were able to

confirm the above results on infants, it is possible that the latter workers may have been dealing with a nonspecific type of skin disorder.

Two groups of workers (Faber and Roberts, 1935; Cornbleet and Pace, 1935) have correlated the appearance of eczematous symptoms in adult subjects with the reduction in the level of unsaturated fatty acids in the blood, and especially with that of linoleate and arachidonate. When the skin condition was relieved by the administration of lard, the improved clinical condition was reflected by an increased plasma linoleate, although the plasma arachidonate was not necessarily augmented (Finnerud *et al.*, 1941). Brown and Hansen (1937) reported the following average values for linoleate in per cent of total acids in young and in adult eczematous patients, respectively: *young patients*, 4.80% and 3.20%; *adult patients*, 5.20% and 4.20%. The mean blood arachidonate of these groups also was lower in eczema, being as follows: *young patients*, 2.83% and 1.34%; *adult patients*, 2.90% and 1.60%. In a comprehensive study of the polyunsaturated fatty acid levels in the blood of 93 well-nourished infants and children, Wiese and her co-workers (1954) observed a slightly lower value for dienoic and tetraenoic acids in the infants than in children 2 to 15 years of age. When 3% of the total calories were ingested in the form of linoleate, 30.3%, 1.5%, and 10.2% of the total plasma fatty acids consisted of dienoic, trienoic, and tetraenoic acids, respectively. Hansen and Wiese (1954) reported that no significant differences in the values of the total plasma fatty acids were to be noted in the case of 57 poorly nourished infants and children, as compared with the figures obtained for well-nourished subjects. However, it was observed that the levels of dienoic, tetraenoic, and hexaenoic acids were significantly lower in the poorly nourished individuals as compared with the well-nourished group. There would thus appear to be a correlation between the EFA content of the blood and the occurrence of eczema, not only in the case of infants but also in that of young children and adults.

In spite of the unequivocal relationship between the skin symptoms and the blood level of EFA in man, it has not been possible to produce the fat-deficiency syndrome in man experimentally by diet. In a single experiment on a normal male subject maintained on a fat-free regimen for six months, Brown *et al.* (1938) were unable to observe the appearance of any of the classical symptoms of fat deficiency. However, a 50% reduction in plasma linoleate and plasma arachidonate occurred, which was completely out of proportion to the extent of decrease of other blood lipids. In spite of this single negative result on man, it would seem to the reviewers that the proof of the requirement of EFA by man is unequivocal. Because of the greater life span, or because of the lower requirement for EFA, the fat-deficiency syndrome cannot be initiated as

easily in man as in the lower animals. Man should be classified with the hog as being less susceptible to fat deficiency, in contrast to the ready production of the fat-deficiency syndrome in the mouse, rat, chicken, dog, and calf.

2. The Effect of Sex

It is a well-known phenomenon that a sex difference exists in the metabolism of fats. This variation is reflected not only by the higher level of ketonuria which obtains during fasting in women as compared with men (Deuel and Gulick, 1932), but also in the increased levels of urine ketone bodies in the female rat in exogenous ketonuria (Butts and Deuel, 1933) and in endogenous ketonuria (Deuel, Hallman, and Murray, 1937), as compared with the levels in male rats. For a further discussion of these and other aspects of sex difference in fat metabolism, the reader is referred to other publications of one of the reviewers (Deuel, 1954a,b; 1955).

It is not surprising, therefore, to note that the optimum requirement for linoleate varies with sex. Data on the requirement of EFA for growth are available only for the rat. If one accepts maximum gain-in-weight during the assay period as an index of optimum nutrition, then one must conclude that the daily requirement for male rats probably exceeds 200 mg. This conclusion was reached on the basis that the curve obtained, when the gain-in-weight is plotted against log dose, is a straight line not only for several daily dosages up to 100 mg. (Greenberg *et al.*, 1950, 1951a), but even for 200 mg. (Deuel, Greenberg, Anisfeld, and Melnick, 1951).

In contradistinction to these results, on the basis of similar experimental data, the daily requirement of the female rat for EFA does not exceed 100 mg. and probably varies between 20 and 50 mg. (Greenberg *et al.*, 1950; Anisfeld *et al.*, 1951). Anisfeld and her co-workers (1951) likewise reported that the reduced growth response of the female rat to increasing doses of linoleate is not related to variations in the tocopherol requirement of the sexes. Cheng, Kryder *et al.* (1952) also noted that sex plays a role in the protective effect of fats against X-irradiation injury. Thus, the male can be protected by fats from X-irradiation under conditions which afford no protection for the female. Although all of the data concerning sex differences in requirement for EFA have been obtained on the rat, there is no reason to believe that the sex factor is unique for this species. One must come to this conclusion if one accepts the fact that this is only one of the expressions of sex variation in fat metabolism—a phenomenon which has been shown to be shared by many species, including man.

3. The Effect of Substances Fed Concomitantly

a. Fatty Acids. The fat-deficiency syndrome has been found to develop much more rapidly when saturated fatty acids are fed to the rat along with the EFA-deficient diet. Evans and Lepkovsky reported, as early as 1932(b), that the fat-deficiency syndrome developed more rapidly in rats on an EFA-free diet than in animals on a similar diet devoid of the saturated fat. These findings were confirmed by Sinclair (1936) and by Deuel, Greenberg, Anisfeld, and Melnick (1951). In a later study by one of the reviewers (Deuel, Alfin-Slater, Wells *et al.*, 1955), it was noted that not only was the time of depletion appreciably shortened when hydrogenated coconut oil was included in the EFA-free diet, but also the level at which the body weight of the rats reached a plateau was considerably lower when hydrogenated coconut oil was present in the diet. However, the continued presence of hydrogenated coconut oil in the diet did not appear to alter the growth response to linoleate, although the animals depleted with the hydrogenated coconut oil diet had a greater growth potential than did those depleted on the fat-free regimen. The intensity of the effect with hydrogenated coconut fat varied with the proportion of this foodstuff included in the diet. Thus, the effect of a 15% addition exceeded that of 5%, and a much more pronounced effect was noted when the fat was included at the 30% level. On the other hand, partially hydrogenated triolein does not influence the length of the depletion period or the body weight at which the fat-deficiency syndrome is established (Wells and Deuel, 1954). Moreover, triolein does not change in any way the effect of linoleate in providing a gain-in-weight in fat-deficient animals. Finally, triolein has likewise been found to have no beneficial or deleterious influence upon the resistance to X-irradiation injury in the rat (Cheng, 1954). It is therefore apparent that hydrogenated coconut oil and partially hydrogenated triolein behave differently in the rat insofar as their relationship to EFA is concerned. It is possible that the effect of the hydrogenated coconut oil may be related to the large proportion of lauric acid and shorter chain acids in its molecule, as compared with the more physiological triolein.

b. Mineral Oil. Bacon and co-workers (1952) demonstrated that a fat-deficiency syndrome could be precipitated in weanling rats within a relatively short period if mineral oil was incorporated into a fat-low diet. When the level of added mineral oil was less than 5%, the cessation of growth was noted at the same time as in the control tests (at 11 to 12 weeks); however, when 7.5% or 10% of mineral oil was included in the regimen fed to the weanling rats, the classical fat-deficiency symptoms

appeared within 2 to 3 weeks. In addition to growth failure, an increased water consumption was likewise noted.

Proof that the deficiency produced by the inclusion of mineral oil in the diet is a fat deficiency was based not only upon the typical symptoms which developed but also upon the fact that the inclusion of 50 mg. of linoleate per day in the diet prevented them. Moreover, there was an increased excretion of fatty acids in the feces of mineral oil-fed rats; in addition, the fecal fatty acids were found to have an increased iodine value after the ingestion of mineral oil. However, it is believed that this effect cannot be ascribed solely to the solvent action of the hydrocarbon on EFA, since the deficiency symptoms were prevented by the administration of linoleate either orally or intraperitoneally.

4. The Effects of Vitamins and Hormones

Although the metabolism of most of the water-soluble B vitamins is interrelated with that of fat in general, it was pointed out by Salmon (1941) that the deficiencies in pantothenic acid, pyridoxine, and EFA, respectively, result in the production of forms of dermatitis which are similar in appearance. The closest relationship appears to exist between the requirement for fat and that of pyridoxine (Hogan and Richardson, 1935; Birch and György, 1936). The dermatitis which occurs on a diet low in pyridoxine but containing 10% of fat can be cured when a small amount of lard is added to the diet (Birch and György, 1936). In fact, Birch alone (1938) later showed that the onset of dermatitis due to a pyridoxine deficiency was delayed by the addition of fat to the diet; in some cases, the abnormal skin condition was completely prevented, even up to the time of death.

Most of the evidence points to the fact that the effectiveness of fats in counteracting the dermatitis caused by pyridoxine deficiency is proportional to the unsaturation, *i.e.*, presumably to the EFA content. This explanation would account for the fact that lard is effective in alleviating pyridoxine deficiency, since it is ordinarily relatively high in arachidonic and linoleic acids, whereas butter, which has a relatively low content of EFA, is somewhat less efficacious. Schneider (1940) ascribed the low antidermatitis potency of rancid butter to the destruction of the antidermatitis factor (later identified as linoleic acid) during the course of rancidification.

All of the principal components of the EFA are capable of curing pyridoxine deficiency. According to Quackenbush *et al.* (1942a) linoleic acid is the most effective of the three principal EFA in clearing up the deficiency symptoms due to the lack of pyridoxine. Salmon (1938) found that the methyl esters of linoleic and linolenic acid are less effective

than the oils or their total fatty acids. Schneider and co-workers (1940) state that linoleic acid possesses a better curative action in pyridoxine deficiency than does linolenic acid; it has also been found that the effectiveness of several natural fats in counteracting acrodynia is in proportion to their linoleate content. Corn oil was found to be superior to linseed oil, while cod-liver oil was almost completely ineffective (Salmon, 1938). Richardson and associates (1941) reported that both methyl arachidonate and linoleic acid resulted in good growth, but that neither afforded permanent protection against the dermatitis caused by pyridoxine deficiency. These workers suggest that the EFA do not replace pyridoxine but simply delay the onset of the skin symptoms.

The question naturally arises as to whether or not the skin condition resulting from EFA and from pyridoxine deficiency is the same. Medes *et al.* (1947) emphasized the fact that an interrelation exists between the two types of deficiency; however, their results might be interpreted to mean that two different factors are involved. When the diet was lacking in both factors, a relief from deficiency symptoms was obtained when either ethyl linoleate or pyridoxine was administered. On the other hand, the effectiveness of ethyl linoleate was less than it was when optimum doses of pyridoxine were given. Best results were obtained when both of these components were included in the diet simultaneously.

The relationship between pyridoxine and oleic acid appears to be opposite to that of pyridoxine and the EFA. Thus, Sarma *et al.* (1947) observed that the growth inhibition in rats caused by feeding diets deficient in pyridoxine or pyridoxal was accentuated when oleic acid was added to the diet. However, the inhibitory action of oleate could be counteracted by the administration of additional quantities of vitamin B_6. Sinclair (1952) has recently reviewed the relationship between pyridoxine and the EFA.

Fat is an effective agent in thyrotoxicosis. Thus, Greenberg and Deuel (1950) observed that, on diets with a high cottonseed oil content, not only did rats not lose weight when thyroid powder was also given, but no mortality occurred. In sharp contrast to this finding, the inclusion of thyroid hormone in a low-fat diet not only caused a marked depression in the growth of the rats but a mortality as high as 83% was observed.

One explanation for the protective effect of fats in thyrotoxicosis is that they furnish the EFA. However, oleic acid, as well as linoleic acid, was found to prevent the increase in metabolism following the administration of thyroid powder (Keeser, 1938). On the other hand, Zain (1936, 1937) reported that linoleic acid, but not stearic acid, prevented the loss of liver glycogen after massive doses of the thyroid hormone. It is of course possible that the failure of stearic acid to prevent thyrotoxicosis

may be related to the fact that it cannot be absorbed. Since a practically fat-free liver preparation has been shown to have a beneficial effect in the fat-deficiency syndrome, Ershoff (1949) is of the opinion that the EFA are not necessarily the specific agents in counteracting thyrotoxicosis. On the other hand, Greenberg (1952) reported that both methyl linoleate and cottonseed oil have a protective effect against thyrotoxicosis.

VI. Interconversions of the Polyunsaturated Fatty Acids

1. Interconversions of Linoleic Acid

a. Conversion to Arachidonic Acid. (1) Experimental evidence of the reaction. Although there are abundant data to indicate that the animal cannot synthesize the so-called EFA *de novo*, there is also considerable evidence that a certain degree of interconversion is possible between the several members of the EFA.

One type of evidence of the interconversion of the polyunsaturated acids is based upon the fact that one acid may prevent the deficiency caused by the exclusion of other essential fatty acids from the diet. Thus, Turpeinen (1938) and Smedley-MacLean and Nunn (1940) are of the opinion that linoleic acid is the precursor of arachidonic acid. The higher biopotency of arachidonic acid as compared with linoleic acid (see Section IV, 5) is explained as due to the fact that linoleate is only partially converted to arachidonic acid; the comparative biopotency of linoleate is a reflection of the amount of arachidonate formed from given amounts of linoleate.

The most convincing proof of the linoleate → arachidonate conversion is based upon balance experiments involving the content of arachidonate in the tissues when linoleate is fed, as compared with the values in control experiments. The supplementation of fat-deficient rats with corn oil (which contains linoleate but no arachidonate) was found to increase markedly the tetraenoic acid content of liver, kidney, heart, and brain (Rieckehoff *et al.*, 1949). Even before the availability of the spectrophotometric method of analysis for the polysaturated acids, Ellis and Isbell (1926a, 1926b) found evidence of the appearance of arachidonic acid in the pig upon the ingestion of linoleic acid. Nunn and Smedley-MacLean (1938), and also Smedley-MacLean and Hume (1941) both presented additional evidence of the appearance of arachidonate in fat-deficient rats following the administration of linoleate. Widmer and Holman (1950), in a study of the effects of fatty acids in the diet on the synthesis of the EFA, confirmed the transformation of linoleate to arachidonate in the rat.

Evidence for the synthesis of arachidonic acid from linoleic acid has likewise been adduced from experiments on chickens. Thus, Reiser and

Gibson (1950b) reported an increase of tetraenoic acid in the tissues of growing chicks on a fat-free diet when cottonseed oil or ethyl linoleate was fed. The increases were least in the neutral fat of the carcass, more pronounced in the organ neutral fat, and increasingly greater in the carcass and organ phospholipids. In other studies (Reiser and Gibson, 1950a; Reiser *et al.*, 1951), it was shown that the polyunsaturated fatty acids reached a minimum value in the yolk of eggs obtained from hens on a fat-free diet. When single unsaturated acids were fed, their interconversion to other unsaturated acids in the egg-yolk fat could be followed. It was found that tetraenoic acid (arachidonic acid) made its appearance in the yolk after ethyl linoleate or cottonseed oil had been fed to the hens. The fact that arachidonic acid is not a constituent of plants but is usually a component of animal tissues (Holman, personal communication, 1955), and that it may substitute for linoleate in the nutrition of the animal, leads one on *a priori* grounds to conclude that the transformation of linoleate to arachidonate can actually occur. Finally, probably the most convincing evidence of the change of linoleate to arachidonate is found in the experiments with tagged acids described in the next section.

(*2*) *Mechanism of the conversion.* The reactions involved in the change of one unsaturated acid to another have been largely a matter of conjecture. Reiser (1951) suggested that "fragments of the ingested acids containing the double bonds might combine to form the more highly unsaturated members of the series." Thomasson (1953) is in agreement with Greenberg (1951), who suggests that two molecules of linoleate give rise to one arachidonic acid molecule. Sinclair (1952) is likewise convinced of the synthesis of arachidonic acid from linoleic acid, "despite the improbability of a change involving the addition of two carbon atoms and desaturation at the 5:6 and 8:9 positions." Although the mechanism of these changes is not entirely understood, Mead *et al.* (1953) have demonstrated it in the intact animal. Thus, it was found that when carboxyl-labeled acetate was injected into weanling rats, it appeared as carboxyl carbon in the arachidonic acid, as demonstrated by analysis of the degraded octabromides prepared from the acids isolated from the tissues.

When the linoleate is altered by conjugation or elaidinization, it cannot be converted to biologically active tetraenoic acids. Thus Reiser (1951) reported that, when conjugated trilinolein was fed to hens, the conjugated dienoic acids appeared in the egg-yolk fat, but that no more highly conjugated acids were observed. Holman (1951a) noted that, when elaidinized linoleic acid was fed to rats, it was converted to spectrophotometrically detectable tetraenoic acid, as demonstrated by alkali isomerization, but the tetraenoic acid was reported to be biologically inert.

b. Conversion to Polyunsaturated Acids Other than Tetraenoic Acid. Although the experimental data are exceedingly fragmentary, the present indication is that a species difference obtains in the possible transformations of the dienoic acids. Thus, on the one hand, Reiser and Gibson (1950b) as well as Reiser *et al.* (1951) found that the laying hen could convert ingested linoleate into pentaenoic acid as well as into tetraenoic acid. However, there was no formation of either trienoic or hexaenoic acids in this species. In contradistinction to these results, the Holman group (Rieckehoff *et al.*, 1949; Widmer and Holman, 1950) found that hexaenoic and pentaenoic acids, as well as tetraenoic acid, arise from dienoic acid in the case of rats; hexaenoic acid was present in the greater amount. Although these workers failed to observe the formation of pentaenoic or hexaenoic acids, after supplementation, when selected tissues were used, evidence for the synthesis of both of these types of acids was obtained when the whole animal was analyzed.

2. Interconversions of Linolenic Acid

a. Factors Altering the Trienoic Acid Content of Tissues. The metabolism of linolenic acid poses certain interesting variations as compared with that of linoleic acid. One of the earliest observations was that this trienoic acid was present in exceedingly small quantities, even when a considerable amount was present in the ration. This led Bloor (1943) to postulate that linolenic acid is preferentially utilized and is not deposited.

However, it now appears that the deposition of linolenic acid in the tissues is dependent upon the species of animal. On the one hand, Ellis and Isbell (1926a,b) reported that only small amounts of trienoic acids were to be found in the case of pigs, even when they were fed large amounts of soybeans. However, Beadle *et al.* (1948) did find as much as 11.4% of this acid in the "yellow" fat of swine. Rats which had received a linseed oil diet were found to have as much as 25.6% of linolenate in their fat depots (Beadle *et al.*, 1948) Brooker and Shorland (1950) reported that linolenate comprises as much as 17% of the fat of pasture-fed horses. In most species, however, linolenate is absent from the depot fat.

The most striking fact is that the proportion of linolenate in the storage fat is *decreased* by feeding polyunsaturated acids and is *increased* by the administration of fat-free diets. Thus, Reiser *et al.* (1951) observed that on normal rations the neutral fat and phospholipid of the egg-yolk fat of hens contained only 0.18% and 0.00%, respectively, of trienoic acids. After 52 weeks on a fat-free diet, the trienoic acid content of the above fractions of egg yolk was found to be 0.42% and 2.3%, respectively. Heart fatty acids, which normally had a content of 22% of trienoic acid, were found to have only about 4% after supplementation with corn oil

or cod-liver oil (Rieckehoff *et al.*, 1949). More recently, Klein and Johnson (1953) noted that the trienoic acid content of the mitochondria, poorly sedimentable layer, and microsomes of the livers of senescent rats and of rats on a fat-free diet, was increased over the control level in both cases. Smedley-MacLean (1943), who had also reported an increase in trienoic acid in fat deficiency, expressed the opinion that the trienoic acid present may be a partially hydrogenated arachidonic acid, resulting from the attempt on the part of the animal to make the most of its stores of arachidonate.

b. Transformations of Administered Linolenate. Reiser and co-workers (1951) reported that the hen is able to convert ingested linolenic acid into dienoic, tetraenoic, pentaenoic, and hexaenoic acids and to deposit these polyunsaturated acids in egg-yolk fat. On the other hand, Holman (1951b) is of the opinion that the rat converts trienoic acid mainly to hexaenoic acid, although in another report (1951a) this author did note the formation of unsaturated acids containing four, five, and six double bonds in the rat after the administration of linolenic acid.

It is difficult to harmonize the above data with the information available on the nutritional value of linolenic acid. A number of workers, including Burr (1942) and Greenberg *et al.* (1950, 1951b) have adduced evidence to show that linoleic and linolenic acids have different functions. Whereas linoleic acid and arachidonic acid can support growth and also alleviate skin symptoms, it is suggested that linolenic acid only supports growth. Holman (1951a,b) believes that the tetraenoic acid produced in the animal as a result of the administration of linolenate is probably an inactive isomer. The whole subject must be considered to be in a state of flux, in view of the recent finding of Thomasson (1953) that γ-isolinolenic acid (6,9,12-octadecatrienoic acid) is the active essential fatty acid (131 U. per gram) and that ordinary linolenic acid (9,12,15-octadecatrienoic acid) possesses no significant biopotency (9 U. per gram).

3. Interconversions of Elaeostearic Acid

Miller and Burr (1937) found that when the conjugated isomer of linolenic acid, namely, elaeostearic acid (9,11,13-octadecatrienoic acid), is given to rats, it rapidly loses its absorption band at 270 mμ, and a new maximum appears at 235 mμ. This is interpreted to mean that one double bond has been saturated, with the resultant formation of a dienoic acid. According to Reiser (1951), a similar change of trienoic acid to dienoic acid occurs in the case of the hen when tung oil, which contains principally α-elaeostearic acid, is fed. In another test of Reiser *et al.* (1951), it was noted that α-elaeostearic acid was transformed to acids having two to six double bonds.

4. Interconversion of the More Highly Unsaturated Acids

Information regarding the changes of the tetraenoic and more highly unsaturated acids is quite limited. Holman and Taylor (1950) presented some evidence that hexaenoic acid is deposited in increased amounts in the heart and brain, and pentaenoic acid in the liver of rats after supplementation with ethyl arachidonate. However, it is questionable whether or not the tetraenoic acid was converted to the more highly unsaturated fatty acids, since there is some indication that impurities in the preparation of arachidonate were sufficient to account for the newly deposited pentaenoic and hexaenoic acids. Reiser and Gibson (1950b) noted that a significant increase in dienoic acid, but no augmentation in trienoic acid, occurred in growing chicks fed cod-liver oil which was free from dienoic and trienoic acids. According to Clément and May (1953), conjugated tetraenoic acid can be hydrogenated by rats to yield conjugated trienoic acid.

Figure 1 presents some known interconversions of the several polyunsaturated acids.

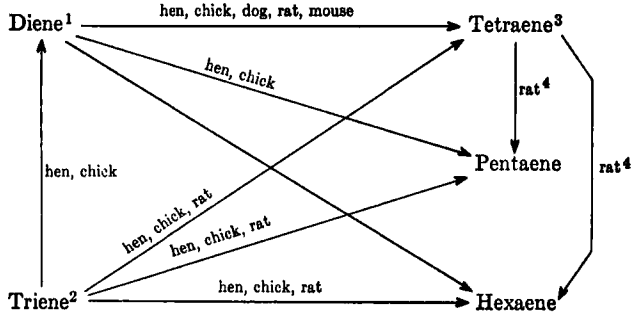

FIG. 1. The biological interconversions of polyunsaturated fatty acids.

1. Dienoic acid is formed from diene-free fatty acids of cod-liver oil, by the chick.
2. Trienoic acid increases upon depletion of other acids, and may be formed from arachidonic acid.
3. The tetraenoic acid formed from trienoic acid appears to be a biologically inactive isomer of arachidonic acid.
4. Uncertain, because the tetraene used was impure.

VII. Physiological Functions Related to the Essential Fatty Acids

1. Growth

The classical demonstration of the necessity of EFA in the diet has been their effect on growth when administered to animals on a fat-free diet. When weanling rats are placed on a fat-free diet, they continue to

grow normally for 2 or 3 weeks, after which the rate of gain-in-weight is decreased below that observed in the case of rats on a diet containing EFA. Within 8 to 10 weeks, the body weights usually reach a plateau, and this may be followed by some decrease in body weight (Deuel, Greenberg, Calbert *et al.*, 1950). When hydrogenated coconut oil is included in the fat-free regimen during the depletion period, the period required for fat depletion is shortened, and the average body weight at which depletion occurs is reduced (Deuel, Alfin-Slater, Wells *et al.*, 1955). Skin symptoms characteristic of the fat-deficiency syndrome invariably develop concomitantly with the retardation in growth. All of these fat-deficiency symptoms can be counteracted when cottonseed oil or other EFA-containing fats are incorporated in the diet; they can also be prevented or cured by the administration of the EFA, either as the free acids or as their esters.

The above results are best explained on the basis that the EFA provide a necessary factor to permit growth, either by furnishing essential building stones in the form of EFA themselves or by providing required components for certain enzyme systems. Smedley-MacLean and Hume (1941) reported that, when the Walker tumor is implanted in rats receiving a fat-free diet, there is a marked decrease in the ratio of highly unsaturated fatty acids to the fat-free dry weight of the subcutaneous tissues. This effect is interpreted as an indication that the EFA are used up in the formation of new tumor tissue; the subcutaneous fat depots are undoubtedly called upon to furnish the extra EFA required. Thus, growth may well be associated with the metabolism of the EFA.

The activity of the cytochrome oxidase system in the liver is markedly increased in rats deficient in EFA (Kunkel and Williams, 1951). These same workers noted a slight decrease in choline oxidase activity, but no alteration was observed in that of succinic oxidase, during the fat-deficiency syndrome. It is suggested that the increased activity of the cytochrome oxidase system may largely account for the augmented level of metabolism in fat deficiency; it will be recalled that Wesson and Burr, as early as 1931, and also Wesson alone (1933) reported that high metabolic rates and high respiratory quotients regularly accompanied the fat-deficiency syndrome. Engel (1942) postulated that, in the absence of the EFA, choline fails to exert its normal lipotropic action. Smedley-MacLean and Nunn (1941) likewise stated that fat cannot be laid down in the tissues on an EFA-deficient diet. This situation likewise would result in the failure of growth.

2. Normal Skin Development

The most consistent and pronounced deficiency symptom which has been observed in all species as a result of fat deficiency is the abnormal

skin condition. This has been described as an eczematous condition in man; in the rat, mouse, dog, and calf it is reflected by a scaliness of the tail, together with roughness of the skin. Scaliness of the paws is another prominent symptom in the case of the rat, mouse, and dog.

The skin symptoms resulting from pyridoxine deficiency and from lack of EFA closely resemble each other. Thus, Birch and György (1936) reported that dermatitis, produced by a low-pyridoxine diet, could be cured when lard was added to the diet. In later work, Birch (1938) demonstrated that the presence of fat in the diet delayed the onset of skin symptoms on a diet low in vitamin B_6; in fact, it was proved that the skin symptoms resulting from pyridoxine deficiency could be prevented by fat feeding even up to the time the animals succumbed from avitaminosis. Quackenbush *et al.* (1942b) noted that, although pyridoxine produced a temporary alleviation, it did not effect a cure. Pantothenic acid was completely without effect in counteracting the symptoms produced by fat deficiency.

Linoleic and arachidonic acids are the most effective of the acids in clearing up the skin symptoms resulting from pyridoxine deficiency or from fat deficiency. Quackenbush and co-workers (1942a) are of the opinion that linoleic acid is more effective than linolenic acid as an antidermatitis factor. In line with this result, Burr *et al.* (1940) reported that corn oil (which has no linolenic acid) is more potent in preventing the skin disorders of fat-deficient animals than is linseed oil (which is relatively rich in linolenic acid at the expense of linoleic acid). In fact, the ability of natural fats to counteract the skin disorders has been found to be in proportion to the linoleic acid content of the diet. According to Richardson *et al.* (1941), methyl arachidonate and methyl linoleate are equally effective in the treatment of the dermatitis produced by vitamin B_6 deficiency.

In a comprehensive study of skin structure as related to diet, Hansen *et al.* (1951) proved that the absence of fat in the diet of dogs brings about definite alterations in the epidermis and dermis, including the hair follicles, the sebaceous glands, and the capillaries. Wiese and Hansen (1951) observed that the nutritional status of dogs which were maintained on different levels and kinds of fat could be assessed from the level of serum linoleate and serum arachidonate. In a later study, Hansen and associates (1954) postulated that dietary fat supplies a factor necessary for the maturation of epithelial, sebaceous, and sudoriparous cells. When fat is absent from the diet of either puppies or adult dogs, distinct skin abnormalities obtain, which can be recognized both microscopically and macroscopically. When EFA are available, the changes occur in the reverse order. The alterations in the stratum corneum, the collagen struc-

ture, and the cellular infiltration of the dermis, the changes in hair follicles, in sebaceous glands, and in sudoriparous glands are all related to changes in the epidermis. The most rapid restoration of the normal microscopic structure was noted when the dietary fat was high in unsaturated fatty acids. The specificity of the skin picture in EFA deficiency was demonstrated by Ramalingaswami and Sinclair (1953) in rats; thus, the histological picture of the skin in EFA deficiency closely resembled that of phrynoderma, whereas the microscopic skin changes in vitamin A deficiency bore only a slight resemblance to this condition.

3. Pregnancy and Lactation

There is considerable evidence that the level of dietary fat may be of considerable importance in physiologic functions such as pregnancy and lactation. It is now certain that at least a portion of the beneficial effect of fats on pregnancy and lactation is to be ascribed to their content of EFA.

Evans and collaborators (1934a,b) were the first to prove that normal reproduction is impossible when the EFA are absent from the diet. The addition of saturated acids to fat-free diets failed to alleviate the symptoms (Evans *et al.*, 1934b). The mothers were unable to suckle their young in the absence of the required nutrients; male rats were also found to become sterile on the fat-free diets (Evans *et al.*, 1934c). The addition of small amounts of EFA to such fat-free regimens was shown to cure the difficulties in reproduction, as well as to prevent their development.

The more recent studies have contributed some information as to the quantitative aspects of EFA required for pregnancy and lactation. Quackenbush *et al.* (1942a) noted that the quantity of EFA required for reproduction in rats was approximately twice that necessary to cure the dermal lesions arising on a fat-free diet. Ordinary linolenic acid (9,12,15-octadecatrienoic acid) was found to be relatively ineffective in permitting normal reproduction. As far as the reviewers are aware, there is no information as to whether or not γ-linolenic acid (6,9,12-octadecatrienoic acid), which Thomasson (1953) reported to be as active as linoleic acid in promoting growth on a fat-free diet, is effective in allowing normal reproduction. Linoleic and arachidonic acids have about the same biopotency insofar as reproduction is concerned (Quackenbush *et al.*, 1942a). Deuel, Martin, and Alfin-Slater (1954b) gave data on pregnancy and lactation of female rats which were supplemented with several levels of cottonseed oil or of methyl linoleate during the period of breeding and lactation. Although fat was not required by the female rat for conception, when the diet was otherwise complete, the pups were invariably born dead or died immediately after birth. It was found that, whereas

daily doses of cottonseed oil as low as 10 mg. insured the survival of the pups for 3 days, in some cases as much as 200 mg. of this fat were required per day for an optimum effect. Since comparable results were observed when 80 mg. of methyl linoleate were fed, it was concluded that the EFA constitute the protective component in fat. The requirement of the female rat for EFA during pregnancy and lactation is as high, if not higher, than the optimum required for growth in this sex.

From the standpoint of lactation performance, fat has long been known to stimulate this function (Maynard and McCay, 1929; McCay and Maynard, 1931; Maynard and Rasmussen, 1942). Although Loosli *et al.* (1944) did observe an improved lactation performance of rats on diets containing corn oil, as compared with animals on a fat-free diet or on one containing hydrogenated coconut oil, which might be interpreted as related to an intake of EFA, no improvement in lactation was noted when as much as 125 mg. of linoleate was given per day to the mother or directly to the pups. However, in the most recent studies of Deuel, Martin, and Alfin-Slater (1954b) they found that 100% mortality obtained before weaning when the fat-free diet of the mothers was supplemented with 10 mg. of cottonseed oil or with 10 mg. of linoleate. However, the lowest mortality rate and the highest weaning weights of the pups were noted in rats receiving 100 or 200 mg. of cottonseed oil daily, or 80 mg. of linoleate. On this basis, the latter workers ascribe the beneficial effect of fat on reproduction and also on lactation to the EFA present in this foodstuff.

4. Protection against X-Irradiation

Cheng and her co-workers (1952) reported that cottonseed oil in amounts as low as 2% in the diet of male rats affords protection against multiple sublethal doses of X-ray, as compared with the situation noted in rats on a fat-free diet. Decker *et al.* (1950) had observed earlier that a typical EFA deficiency could be provoked in fat-depleted mice when they were exposed to X-irradiation. Although cottonseed oil had a protective effect against X-irradiation in the experiments of Cheng *et al.* (1952), irrespective of whether old rats or young mature male animals were employed in the tests, it was found that fat protected only the old female rats against X-irradiation. It was suggested that, because of the higher linoleate requirement of the male animals, they were depleted of EFA sooner on the fat-free diet than were the female rats. Consequently, the EFA level was not the limiting factor in the protection of young rats. This hypothesis was supported by the longer survival of the control females than of the control males in the group of young mature animals. In another study, it was found that the linoleate protection was in addi-

tion to that afforded by liver powder; moreover, hydrogenated coconut oil was found to yield no protection (Cheng and Deuel, 1953).

In subsequent studies (Deuel, Cheng *et al.*, 1953), it was proved that the protective effect of cottonseed oil was a function of the EFA present therein. Thus, when 10 mg. of ethyl linoleate were given daily to fat-depleted rats which were subsequently exposed to weekly doses of X-rays of 300 r, a significantly greater survival rate was noted in the case of the supplemented rats, despite the fact that the linoleate-treated animals received as much as 45% more X-irradiation than the control rats did. More recently, Cheng, Ryan *et al.* (1954b) have established the fact that the optimum daily protective dose of linoleate for male rats against X-irradiation injury probably exceeds 100 mg. Thus, it was demonstrated that the average survival period for LD_{25}, LD_{50}, and LD_{75} were progressively greater when the protective dosages of linoleate were 10, 50, and 100 mg. per day. The beneficial effect of fat in protection from injury due to X-irradiation has been shown to obtain with widely varying doses of X-ray and with considerable variations in the frequency with which it was applied (Cheng, Alfin-Slater, and Deuel, 1954a). Linolenic acid was recently shown to afford only slight protection against X-irradiation when given in doses as high as 80 mg. per day to male rats (Cheng *et al.*, 1955); on the other hand, when linoleate was administered simultaneously with linolenate, a marked synergism was noted, as also obtains with growth (Greenberg *et al.*, 1950).

The reason for the beneficial effect of the EFA against X-irradiation damage can only be conjectured. Since there is some indication that linoleate is required for the growth of new tissues, as well as for the repair of damaged ones, the restitution of such injured tissues would proceed more rapidly in the cases in which an adequate supply of EFA was available. The primary tissue injured by exposure to X-rays under most conditions is the skin. Since it is known that the EFA are concentrated in the subcutaneous fat, the protective effect of linoleate may be ascribable to the rapid local healing afforded in the subcutaneous tissues; the variation in time required for this process to take place with a high EFA storage, as compared with that in an EFA-deficient animal, may spell the difference in survival in the presence and absence of a fat which contains EFA.

5. Relation to Capillary Fragility

Kramár and Kovács (1939) were the first to call attention to the fact that the capillary resistance of human subjects, especially of those with allergic manifestations, was higher in patients who had received vegetable oils than in those who had partaken exclusively of a diet containing animal fats. In studies on rats 14 years later, Kramár and Levine (1953)

were able to prove decisively that the resistance of the skin capillaries of immature rats, fed a diet devoid of fats or fatty acids, or one containing 5% of methyl stearate, began to decrease at 50 days, and soon reached pathologically low levels. This change in capillary resistance was shown to be inversely proportional to the increase in capillary permeability. This increased capillary permeability was found to occur simultaneously with the flattening of the growth curve, but before other symptoms of EFA deficiency were evident. Caudal necrosis did not occur.

The low capillary resistance of the fat-deficient rat could be restored to normal by the administration of small amounts of linseed oil or of linoleic acid itself. It would appear that one must consider decreased capillary resistance (or increased capillary permeability) as an additional manifestation of the fat-deficiency syndrome in rats and also in man.

6. Relation to Cholesterol Metabolism

Fats have been widely maligned as a food in recent years, both as a carrier of cholesterol (animal fats) and as a source of endogenous cholesterol (animal and vegetable fats). Since the acetate molecule is known to be incorporated to a large extent in newly synthesized cholesterol, and because acetate is formed on the oxidation of the fatty acid molecule, it has been suggested that the ingestion of fat stimulates the synthesis of cholesterol. However, the rate of synthesis of cholesterol is independent of the amount of fat available; furthermore, the acetate required in this reaction can originate from dietary carbohydrate, either directly from the ingested carbohydrate, or indirectly from the fat formed from it. In order to prevent an overproduction of cholesterol, because of its supposed relationship to arteriosclerosis, diets have been recommended for patients which are practically devoid of all fats.

It has been recently reported, in the case of the rat, that the administration of a fat-free diet does result in a reduction of blood cholesterol; however, there is a concomitant increase of liver and adrenal cholesterol, which may reach twice the normal level (Alfin-Slater *et al.*, 1954a,b). The normal liver cholesterol, 2.06 mg. per gram liver, which was found in the group of rats receiving the diet containing 12.5% of cottonseed oil, compares with averages of 3.60 mg. per gram in one group on the fat-free regimen, and of 4.48 mg. per gram in another group which received no fat. On the other hand, the plasma cholesterol was only 44.4 and 43.0 mg. % in the case of the groups on the fat-free diets, whereas the average value for the group receiving 12.5% cottonseed oil was 64.4 mg. %; this compares with the normal value generally obtained on fat-containing diets devoid of cholesterol. In a later study with weanling rats (Deuel, Alfin-Slater, Wells, *et al.*, 1955), it was observed that the increased level

of liver cholesterol and the decreased figure for blood cholesterol develop within 1 week after the animals are placed on a fat-free diet; the divergence continues and increases during the subsequent 13-week period during which the test is continued.

The role of linoleate in cholesterol deposition and transport is not entirely clear. Kelsey and Longenecker (1941) proved that 62% of the plasma cholesterol of cattle occurred in combination with linoleate. It is only natural to postulate that, in the absence of EFA, cholesterol is deposited in the liver, because there is insufficient linoleate available to transport it to other tissues for metabolism and excretion. However, it has been shown that, in such conditions, the increased cholesterol is deposited in the liver as an ester. The cholesterol esters in the liver of rats have been proved to consist almost entirely of those of saturated and oleic acids; only approximately 10% of the cholesterol occurs in combination with linoleic acid, irrespective of whether or not the diet contains EFA (Achaya *et al.*, 1954a). It would thus appear that linoleic acid is of prime importance in the control of the distribution and deposition of cholesterol in the rat. Whether or not the same situation obtains in the case of man is a moot question.

The results of Cochrane *et al.* (1953), of Kinsell *et al.* (1953), and of Kinsell alone (1954) do indicate that a profound relationship may exist in man between the intake of EFA and the level of plasma cholesterol. These workers have reported that elevated levels of blood cholesterol in conditions such as diabetes can be gradually decreased to normal when large amounts of vegetable fats are included in the diet. On the other hand, this pattern was immediately reversed when animal fats, for instance egg yolk, were taken. The latter effect was not believed to be ascribable to the cholesterol present in the ingested animal fats. Although it is uncertain whether or not the vegetable oil effect is to be attributed to the EFA, it is only natural to assume that the variation in the response of the blood cholesterol to vegetable and animal fats is to be traced to this variation in composition.

The importance of the essential fatty acids in relation to atherosclerosis in the rabbit has recently been clearly demonstrated by Kritchevsky and co-workers (1954). Rabbits were fed complete diets containing the following varied lipid components: I, no fats or cholesterol (control group); II, cholesterol (3%), corn oil (9%); III, cholesterol (3%), shortening (9%); IV, cholesterol (3%); V, corn oil (9%); VI, shortening (9%). The corn oil had an iodine value of 130, and the shortening was a vegetable oil hydrogenated to give a solid fat having an iodine number of 72. After 2 months, the rabbits were sacrificed and the extent of atherosclerosis was given a mathematical score based on 0, +, ++,

+++, and ++++ being considered as 0, 1, 2, 3, and 4. The following averages were obtained on the state of the atherosclerosis in the several groups: I, 0.06; II, 2.71; III, 3.71; IV, 3.80; V, 0.10; and VI, 0.10. It is thus evident that the feeding of corn oil, which contains a high proportion of essential fatty acids, is able to suppress the deposition of cholesterol in the aorta of rabbits to a marked extent, even when the animals are subjected to such a severe strain as is afforded by the presence of 3% cholesterol in the diet. On the other hand, the shortening did not appear to alleviate the condition. These data likewise refute the concept that fat *per se* has an atherogenic effect.

VIII. Distribution of Essential Fatty Acids

1. Distribution in Foods

a. Introduction. The most concentrated sources of the EFA are to be found in vegetable fats. Linoleic acid is the chief representative of this group in the vegetable fats although, in some cases, such as in linseed oil, appreciable amounts of linolenic acid may occur. Arachidonic acid, on the other hand, is never found in vegetable fats. In contradistinction to the relatively high content of EFA in most vegetable fats, the proportion in animal fats is much lower, and, in many cases, practically none are present. Although arachidonic acid is frequently present in animal fats, linoleic acid may be present in a much larger proportion in these fats. Moreover, after a high intake of linolenate-containing fats, this trienoic acid may also appear in animal fats. For a more complete treatment of this topic, the reader is referred to two recent reviews of one of the authors (Deuel, 1954a,b).

b. Presence in Vegetable Fats. As noted above, the vegetable fats have the highest EFA content of any of the natural or processed fats. The linoleate content of oils and fats in this group, which has been verified by bio-assay, include the following: safflower-seed oil, 78.0%; sunflower-seed oil, 68.0%; soybean oil, 58.8% linoleate and 8.1% linolenate; cotton-seed oil, 50.4%; sesame oil, 40.4%; peanut oil, 27.4%; and olive oil, 15.0%. Other vegetable fats, which have a high linoleate content but in which the activity has not been confirmed by bio-assay, include the following: walnut oil, 75.5% linoleate and 10.0% linolenate; hempseed oil, 68.8% linoleate and 24.3% linolenate; poppy-seed oil, 62.2%; and corn oil, 39.1%.

In the case of linseed oil and of several other vegetable fats, a poor agreement has been noted between the results recorded spectrophotometrically and by bio-assay. Thus, although linoleate values as high as 46.7% and figures for linolenate in some cases as high as 60.9% have been

recorded for linseed oil, the bio-assay values of two samples reported by Thomasson (1953) were only 11.9% and 25.6%. In the case of cocoa butter, the bio-assay value is given as 2.2% as compared with a figure of 21.1% determined spectrophotometrically. Coconut (and especially hydrogenated coconut oil) and castor oils have been reported as having practically no essential fatty acids, as determined by spectrophotometric and bio-assay procedures.

c. Presence in Animal Fats. The quantity of EFA in animal fats is largely a matter of the species from which the fat was obtained; also, in cases in which the EFA can be stored, the proportion in the fat varies with the previous diet of the animal.

The animal fats which may contain relatively high amounts of EFA include that from the hog, from the fowl, and especially that in the egg. Thus, on a hempseed oil diet, egg-yolk fat contained as much as 41.9% of linoleate and 10% of linolenate. Likewise, pig fat was reported as having as much as 38.9% of linoleate and 0.5% of linolenate following a soybean oil diet (Deuel, 1954a). However, these high values have not been confirmed by bio-assay. Thomasson (1953) reported a value of only 6.9% for lard when tested by the latter procedure.

In contradistinction to the relatively high content of EFA in chicken and hog fat, the storage fat of beef cattle and of sheep represents only a minimum level of EFA. Although ox depot fat has been reported to have 5.3% of linoleate and 0.5% of arachidonate, Thomasson (1953), employing a bio-assay procedure, obtained a figure of only 1.5% for beef suet; sheep depot fat has been reported to have 5% of linoleate, but this has not been confirmed by bio-assay. More precise data, which have been confirmed by bio-assay, are needed in the case of animal fats.

d. Presence in Hydrogenated Fats. When fats are completely hydrogenated, as, for example, coconut oil, they obviously lose all their EFA. On the other hand, in the case of the vegetable fats which have been partially hydrogenated for the preparation of margarines and shortenings, appreciable quantities of the EFA may remain. One assay, which is based upon a new chemical "isolation" method of Simmons and Quackenbush (1953), assigned a value of 20.5% for the linoleate content of a common shortening (*Primex*). On the other hand, Deuel, Greenberg, Anisfeld, and Melnick (1951) recorded values of 4.2% to 5.9% for the EFA content of different samples of a hydrogenated vegetable margarine, whereas the value for a shortening prepared by selective hydrogenation was 2.7% and that for one prepared by nonselective hydrogenation was 13.2%. It seems probable that the newer margarines on the market, which have greater plasticity and which are blended hydrogenated fats (rather than fats in which the whole sample has been subjected to a uniform hydrogenation),

may be expected to have an appreciably higher EFA content than the former types.

e. Presence in Butters. The EFA content in butters is the lowest in any of the several groups of fats. The bio-assay values reported by Thomasson (1953) for three samples of summer butter were 0.6%, 1.3%, and 1.3%, and the figures reported by one of the authors (Deuel, Greenberg, Anisfeld, and Melnick 1951) for two butters from the New York City market with the lowest and highest iodine numbers of a number of different products investigated were 2.5% and 3.8%, respectively. It would be of importance to know to what extent the EFA content of butters can be influenced by dietary factors.

2. Distribution in Animal Tissues

The quantity of the several EFA present in the tissues depends to a considerable extent upon the dietary conditions. This is particularly true in the case of the blood. Thus, Wiese *et al.* (1954) have shown that the average EFA content of the blood of 60 healthy children who had partaken of diets containing approximately 3% of the total calories as linoleate was as follows, in percentages of the total fatty acids: dienoic acids, 30.3%; trienoic acids, 1.5%; and tetraenoic acids, 10.2%. The value for hexaenoic acid in the serum of healthy children was variable. These workers state that the role of the pentaenoic and hexaenoic acids, if any, is not known. On the other hand, Hansen and Wiese (1954) were unable to find any significant differences in the total fatty acids in the blood of poorly nourished children as compared with well-nourished individuals. However, the dienoic, tetraenoic, and hexaenoic acid levels were distinctly lower, and the proportion of trienoic acid was higher, in the poorly nourished group as compared with the well-nourished children. The following figures were reported for 60 well-nourished and 34 malnourished children, respectively: total fatty acids, 301 and 278 mg. %; dienoic acids, 30.3% and 12.0%; trienoic acids, 1.5% and 2.4%; tetraenoic acids, 10.2% and 7.0%; and hexaenoic acids, 4.2% and 1.8%.

The polyunsaturated fatty acids have a wide distribution in tissues; however, because of the relatively difficult procedures for isolation, and because of the low concentration, there is little information in this field. Arachidonic acid, either as its polybromide or as the pure acid, has been isolated from a number of the tissues. These include the following: liver lecithin (Levene and Simms, 1922); brain phospholipids (Levene and Rolf, 1922a); corpus luteum (Cartland and Hart, 1925); egg yolk (Levene and Rolf, 1922b); pig liver (Brown, 1928); beef adrenal phosphatides obtained by debromination of methyl octabromoarachidate (Ault and

Brown, 1934b); and by the use of chromatography (White and Brown, 1948; Herb *et al.*, 1951a,b).

Holman and Greenberg (1953) recently investigated the proportion of tetraenoic, pentaenoic, and hexaenoic acids in several nonadipose tissues from lambs, hogs, and beef cattle. The highest proportion of arachidonic acid was found in the testes and ovaries. Lamb testes, for example, contained 15.6% of hexaenoic acid and 10.3% of arachidonic acid. From the standpoint of isolation, the most practical sources of hexaenoic acid were beef testes lipid and hog brain lipid, while arachidonic acid could best be prepared from hog liver lipid.

IX. Conclusions

The essential fatty acids, linoleic, linolenic, and arachidonic acids, have been found to mediate a number of reactions in the animal body. Thus, not only are they important in growth and in the nutrition of the skin, as has been recognized since the discovery of their indispensability, but they are now recognized as specific agents in protecting the animal against X-irradiation injury; they also maintain capillary pressure in the subcutaneous blood vessels. The EFA are required for successful pregnancy and lactation. It has recently been shown that the essential fatty acids are necessary for normal cholesterol transport and metabolism in the rat. In the absence of fats from the diet, the cholesterol content in the liver and adrenal tissue reaches abnormally high values; it promptly returns to normal when fats containing the EFA, or the EFA themselves, are added to the diet. The requirement for EFA in the rat has been shown to be a sex function; apparently much larger amounts are required for optimum growth by the male than by the female rat. One interesting recent report, which has not as yet been confirmed, is the finding that γ-linolenic acid, 6,9,12-octadecatrienoic acid, is the active isomer of linolenic acid in causing growth, rather than ordinary linolenic acid, 9,12,15-octadecatrienoic acid, as was formerly believed.

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