trienes. On this basis, oleic greatly outranks palmitoleic acid as a precursor. Mead (21) suggested that the enzyme systems are not prominently selective since the ratios oleic:palmitoleic were in his experiments of the same magnitude as those of 5,8,11–20:3 : 7,10,13–20:3. Klenk and Tschöpe (18) referred to the acids of rat liver phosphatides in EFA-deficient rats and found a ratio of about 3:1 for the polyenoic acids of oleic and palmitoleic type.

In a broader comparison of monoenoic acids it is well known that Δ^{odd} monoenes are by far prominent in amts above Δ^{even} and that their conversion products play a much greater role. One-carbon degradation of fatty acids has been demonstrated for certain animal tissues and compositional data suggests that it applies also to unsaturated acids (17). In such case, it leads from Δ^{odd} to Δ^{even} acids and explains in all likelihood the occurrence of 8–17:1 in the rat. However, one-carbon degradation has little importance for the composition of the total rat fat.

The preference for biosynthesis of Δ^{odd} monoenes extends to odd-numbered acids for which those of mullet oil are a recently reported example. Among the unsaturated C_{15}, C_{17} and C_{19} acids of this fish oil, neither Δ^{even} monoenoic nor any polyenoic acids possibly derived from them have been found in distinct amts (33).

Biological pathways other than desaturation of saturated long-chain acids lead also to Δ^{odd} monoenes. Such direct synthesis of monoenoic acids has been shown for some micro-organisms and 10–16:1 (in *Mycobacterium phlei*) is the only exception known to us where a Δ^{even} monoene represents an appreciable portion of the acids (19). The occurrence of a Δ^{even} monoene, 6–16:1, in the rat is surprising. However, Kishimoto and Radin (17) found 6–16:1 to be a significant portion of 16:1 isomers from pig brain. The authors concluded that there is a mechanism for synthesis of acids unsaturated in position 6 which so far had not been recognized in animals, and our findings indicate that such synthesis is not limited to brain tissue.

Neither 10–18:1 nor 10–16:1 were found in rats, nor have they been identified from pig brain (17). Very minor amts of 10–20:1 and 10–22:1 are the only Δ^{even} monoenes of even-numbered chain length identified in the latter. Strong preference for desaturating carbons 9 and 10 prevails above desaturation of the neighboring carbons 10 and 11 and minimizes the same for carbons 8 and 9. The zig-zag form of the aliphatic chain may not permit a suitable steric conformation of these carbon atoms in reference to the carboxyl group or its coenzyme A ester.

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REFERENCES

1. Beare, J. L., E. R. W. Gregory, D. M. Smith and J. A. Campbell, Can. J. Biochem. Physiol. *39*, 195–201 (1961).
2. Benjamin, W., A. Gellhorn, M. Wagner and H. Kundel, Am. J. Physiol. *201*, 540–546 (1961).
3. Burr, G. O., and M. M. Burr, J. Biol. Chem. *82*, 345–367 (1929).
4. Butterfield, R. O., C. R. Scholfield and H. J. Dutton, JAOCS *41*, 397–400 (1947).
5. Carroll, K. K., JAOCS, in press.
6. Cuthbertson, F. J., Proc. Nutr. Soc. (Engl. & Scot.) *16*, 70–76 (1957).
7. Dils, R., and G. Popjak, Biochem. J. *83*, 41–51 (1962).
8. Fulco, A. J., and J. F. Mead, J. Biol. Chem. *234*, 1411–1416 (1959).
9. Fulco, A. J., and J. F. Mead, *Ibid*, *235*, 3379–3384 (1960).
10. Garton, G. A., J. Lipid Res. *4*, 237–254 (1963).
11. Gellerman, J. L., and H. Schlenk, J. Protozool., in press.
12. Gellhorn, A., W. Benjamin and M. Wagner, J. Lipid Res. *3*, 314–319 (1962).
13. Glass, R. L., Ph.D. Thesis, Univ. of Minn., 1956.
14. Holloway, P. W., R. Peluffo and S. J. Wakil, Biochem. Biophys. Res. Commun. *12*, 300–304 (1963).
15. Holloway, P. W., and S. J. Wakil, J. Biol. Chem. *239*, 2489–2495 (1964).
16. Holman, R. T., J. Nutr. *70*, 405–410 (1960).
17. Kishimoto, Y., and N. S. Radin, J. Lipid Res. *5*, 98–102 (1964).
18. Klenk, E., and G. Tschöpe, Hoppe-Seyler's Z. Physiol. Chem. *334*, 193–200 (1963).
19. Lennarz, W. J., G. Scheuerbrandt and K. Bloch, J. Biol. Chem. *237*, 664–671 (1962).
20. Luckey, T. D., T. J. Mende and J. Pleasants, J. Nutr. *54*, 345–359 (1954).
21. Mead, J. F., Fed. Proc. *20*, 952–955 (1961).
22. Mead, J. F., and W. H. Slaton, Jr., J. Biol. Chem. *219*, 705–709 (1956).
23. Mohrhauer, H., and R. T. Holman, J. Lipid Res. *4*, 151–159 (1963).
24. Privett, O. S., M. L. Blank and C. Romanus, J. Lipid Res. *4*, 260–265 (1963).
25. Schlenk, H., and J. L. Gellerman, JAOCS *38*, 555–562 (1961).
26. Schlenk, H., and J. L. Gellerman, JAOCS, concurrent publication.
27. Schlenk, H., J. L. Gellerman and D. M. Sand, Anal. Chem. *34*, 1529–1532 (1962).
28. Schlenk, H., and D. M. Sand, Anal. Chem. *34*, 1676 (1962).
29. Schlenk, H., D. M. Sand and N. Sen, AOCS Meeting, Toronto, October, 1962.
30. Schlenk, H., D. M. Sand and N. Sen, Biochim. Biophys. Acta *84*, 361–364 (1964).
31. Schlenk, H., N. Sen and D. M. Sand, Biochim. Biophys. Acta *70*, 708–710 (1964).
32. Scholfield, C. R., E. P. Jones, R. O. Butterfield and H. J. Dutton, Anal. Chem. *35*, 1588–1591 (1963).
33. Sen, N., and H. Schlenk, JAOCS *41*, 241–247 (1964).

Dietary Fat and the Fatty Acid Composition of Tissue Lipids

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Abstract

Some characteristics of the fatty acid composition of animal tissue lipids are described and the origins of tissue fatty acids are discussed briefly. The effect of dietary fat on composition of tissue lipids is discussed. Types of dietary fatty acids for which experimental work is described include polyunsaturated fatty acids, short-chain fatty acids, fatty acids with chain length greater than C_{18}, *trans* unsaturated fatty acids, fatty acids with conjugated double bonds, acetylenic fatty acids, branched-chain fatty acids and oxygenated fatty acids. The individuality of fatty acids is discussed in relation to their roles as components of tissue lipids.

FATTY ACID COMPOSITIONS of animal tissue lipids have been investigated extensively over the past 50 years, first with methods such as fractional crystallization and fractional distillation and more recently by chromatographic techniques. The older analytical work was generally limited to lipids which were available in large quantities and most analyses were made on depot fats although some studies were done on lipids of tissues such as liver and brain (1,2). The advent of newer techniques has made it possible to work with much smaller amts of material. This has resulted in an ever-increasing volume of analytical data, which is no longer restricted to tissues having large quantities of lipids.

The picture that emerges from this wealth of analytical information is one of characteristic fatty acid patterns for the different lipid classes of each tissue. These patterns are subject to alteration by a variety of influences but under ordinary circumstances they are remarkably reproducible in different animals of the same species. Consideration will therefore be given first to some of the characteristic fatty acid patterns found in different lipid classes of the tissues of animals on their normal diets as a preliminary

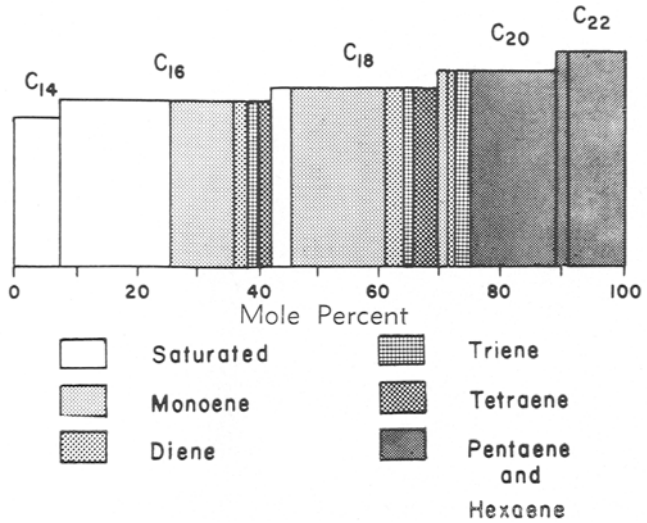

FIG. 1. Bar diagram showing the fatty acid composition of menhaden oil. The width of the bars designates the molar percentage of the fatty acids, and with the height being proportional to the mol wt of the fatty acid esters, the area is proportional to the percentage by weight. (Height is not strictly proportional to mol wt in this diagram since allowance is not made for differences in mol wt due to differences in degree of unsaturation). Open bars are used for saturated fatty acids while shaded bars are used for fatty acids of varying degrees of unsaturation. For simplicity, fatty acids which comprise less than one percent of the total are omitted from this and other similar diagrams.

to discussing changes in the patterns which can be produced by dietary means.

Fatty Acids of Animal Tissue Lipids

Depot Fats

The fatty acids in the fat depots of most animals are present largely in the form of triglycerides. In fish and other forms of aquatic life these fats are characterized by the diversity of fatty acids and particularly by the presence of relatively large amts of unsaturated C_{20} and C_{22} acids (1,3,4). The proportion of saturated fatty acids is correspondingly low and palmitic acid, the main saturated component, rarely exceeds 20% of the total. This is illustrated in Figure 1 by a bar diagram based on data taken from a paper by Ahrens et al. (5), showing the fatty acid composition of menhaden oil.

The depot fats of land animals, in contrast to aquatic species, are relatively simple in composition, consisting mainly of palmitic acid and oleic acid with varying amts of myristic, palmitoleic, stearic, and linoleic acids. There is little if any of the C_{20} and C_{22} fatty acids characteristic of the depot fats of aquatic animals (Fig. 2). Human depot fats are similar to those of other land mammals in respect to fatty acid composition (6–9). Ruminants represent a special class of land animals whose depot fats are modified by the action of the rumen bacteria. These hydrogenate dietary fat and at the same time produce large amts of short-chain fatty acids from carbohydrates and proteins, which are subsequently converted to long-chain acids. The depot fats of ruminants are characterized by relatively large amts of stearic acid which may originate, at least in part, from hydrogenation of other C_{18} acids by rumen bacteria (11–14). A typical pattern is that shown for ox depot fat in Figure 2.

The increasing simplicity in the composition of depot fats as one proceeds up the evolutionary scale led Shorland to suggest that there are phylogenetic

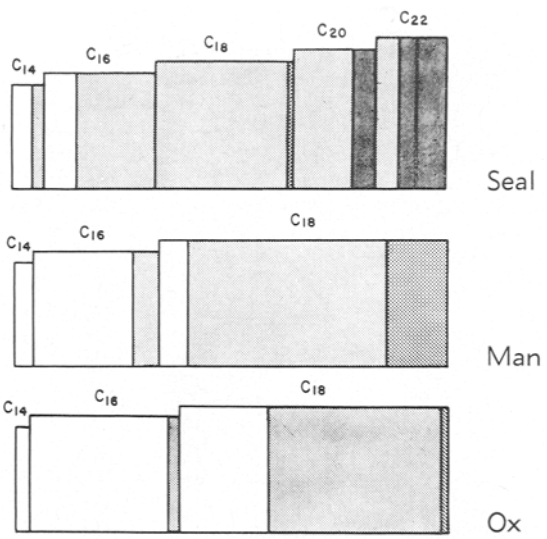

FIG. 2. Fatty acid profiles of seal (10), human (6) and ox depot (1) fats.

differences in the ease with which the fatty acid composition of fat depots can be altered by diet (4, 15,16). According to this concept, lower forms of animal life, such as the fishes, deposit dietary fat relatively unchanged and endogenous synthesis plays only a minor role in determining the fatty acid composition of depot fats. At higher stages of evolution, endogenous synthesis from nonlipid precursors becomes more important and is largely responsible for the simpler fatty acid patterns in the depot fats of higher animals. Although this may be a useful working hypothesis, it does not appear to offer a satisfactory explanation for some of the observed fatty acid patterns in depot fats (17).

Plasma and Liver Lipids

Plasma and liver lipids have been investigated more extensively than those of any other tissues with the possible exception of adipose tissue and brain. Whereas most of the lipid in adipose tissue is in the form of triglycerides, the lipids of liver and plasma contain more phospholipid than triglyceride (Table I). A sizable proportion of the fatty acids of plasma are also in the form of steryl esters, particularly in humans where plasma cholesterol levels are higher than in most other species. It should be recognized, however, that triglycerides contain a higher proportion of fatty acid per unit of weight than either phospholipids or steryl esters.

The fatty acid profiles of liver and plasma triglycerides resemble those of adipose tissue but the phospholipids and steryl esters show significant differences in fatty acid composition (1,6,20–24). This is illustrated for plasma in Figure 3. The main distinguishing features of the phospholipids as compared to triglycerides are the higher levels of stearic and arachidonic acid and the decreased amt of oleic acid. The phospholipids of course comprise a number of different lipid classes and these show individual

TABLE I
Lipid Classes of Liver and Plasma

	Rat liver (18)	Rat plasma (18)	Human plasma (19)
	(Percent of total lipid)		
Steryl esters	3.0	14.6	42.5
Triglycerides	30.5	11.7	12.1
Free fatty acids	0.8	2.9	3.0
Phospholipids	54.3	58.0	31.4
Sterols	11.4	12.8	11.0

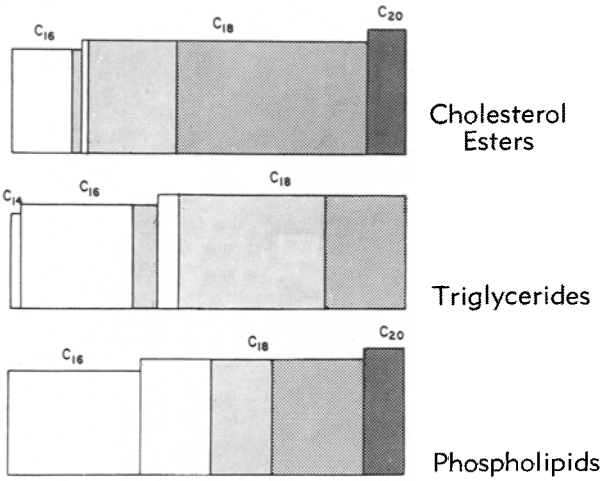

FIG. 3. Fatty acid profiles of cholesteryl esters, triglycerides and phospholipids of human plasma (6).

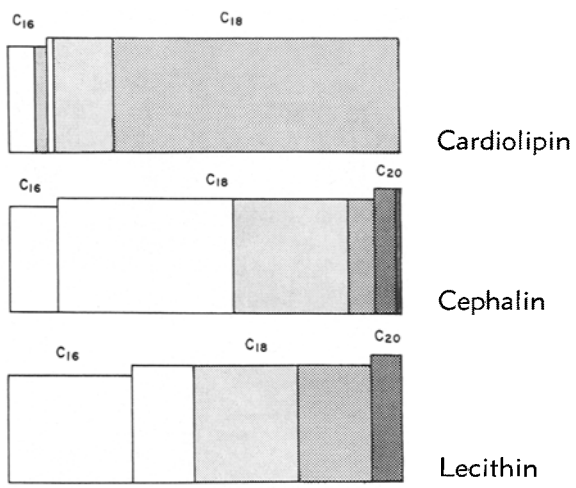

FIG. 4. Fatty acid profiles of different phospholipids of pig kidney (36).

variations in fatty acid composition (25–31). Liver cholesteryl esters are rather similar to the triglycerides in fatty acid composition but plasma cholesteryl esters contain large amts of polyunsaturated fatty acids. In some species (e.g., humans) linoleic acid predominates while in other species such as the rat, arachidonic acid is the main fatty acid (32).

Lipids of Other Tissues

Most studies relating to effects of diet on the composition of tissue lipids have been concerned primarily with the fatty acid composition of plasma, liver or adipose tissue but it is worth noting that analysis of other tissue lipids has provided further evidence of specific distributions of fatty acids in individual lipid classes.

The lipids of brain have been investigated extensively and it is well known that different lipid classes differ greatly in fatty acid composition (33, 35). Fatty acid analyses of individual lipid classes of tissues such as kidney (36,37), heart and skeletal muscle (36,38), adrenal (39,40), and erythrocytes (41–43) have also been carried out with the aid of the newer chromatographic techniques and characteristic features of the fatty acid profiles of different lipid classes are evident. These are illustrated for some of the phospholipid classes of pig kidney in Figure 4. Stearic acid tends to predominate in cephalins while palmitic acid is usually the main saturated fatty acid in lecithins (36–39). Stearic acid reaches even higher proportions in phosphoinositides and gangliosides (25,44,45) and may account for nearly 90% of the total fatty acids of gangliosides. Polyunsaturated fatty acids are not usually found in more than trace amts in sphingolipids. By contrast, unsaturated fatty acids are the main components in cardiolipin with linoleic acid accounting for 60 to 80% of the total fatty acids in cardiolipin isolated from several different sources (36,37). Generally speaking, differences in fatty acid composition between different lipid classes seem to be greater than differences within the same lipid class isolated from different tissues or from different cell fractions of the same tissue (36,46,47).

Intramolecular Distribution of Fatty Acids

In cases where more than one fatty acid forms part of a single lipid molecule, the distribution of fatty acids between the available positions also shows evidence of specificity. The triglycerides of beef, horse, sheep and human fats resemble vegetable fats in having a degree of selective positioning of saturated fatty acids at 1- and 3-positions. The rat and dog have their triglyceride fatty acids distributed in an approximately random fashion while in pig triglycerides the saturated fatty acids are predominantly in the 2-position (48–52).

In lecithins there is also a selective positioning of fatty acids between the α'-and β-positions with saturated fatty acids predominating in the α'-position and unsaturated fatty acids predominating in the β-position (37,53,54). The predominance of unsaturated fatty acids in the 2- or β-position of both triglycerides and phospholipids could indicate a common biosynthetic pathway with α,β-diglycerides as intermediates, as suggested by Kennedy (55), but the fatty acid pattern in phosphatidic acid, which is presumed to be the precursor of the diglyceride, does not seem to fit this concept (37,56), and other factors such as the action of transacylases may be involved (57). In some instances the distribution of fatty acids between different positions of tissue lipid molecules may be simply a reflection of the pattern in dietary fat, since mammalian lipases hydrolyze fatty acids at the 1- and 3-positions preferentially and a large proportion of dietary fat is thought to be absorbed as monoglyceride (58,59). It has been suggested that retention of the β-monoglyceride structure may help to conserve essential fatty acids and protect them from loss by oxidation (60).

When there is a nonrandom distribution of fatty acids at the available sites of attachment in lipids containing two or more fatty acids per molecule, fatty acid analysis of the total lipid fails to give a complete picture of the molecular composition of the lipid class. Analysis of the mixture of fatty acids attached at each particular site is also insufficient because it gives little information on the combinations of fatty acids present in individual lipid molecules. A complete description of such lipid classes presents very difficult problems which can hardly be solved even with the techniques presently available. Methods are being developed, however, in a number of different laboratories (61–66) with the aim of providing this information which is basic to an understanding of the significance of fatty acid composition of tissue lipids.

Sources of the Fatty Acids

The fatty acids in animal tissues may either be derived endogenously by synthesis from small molecule precursors or they may be obtained exogenously from the diet (16). Interconversions of fatty acids

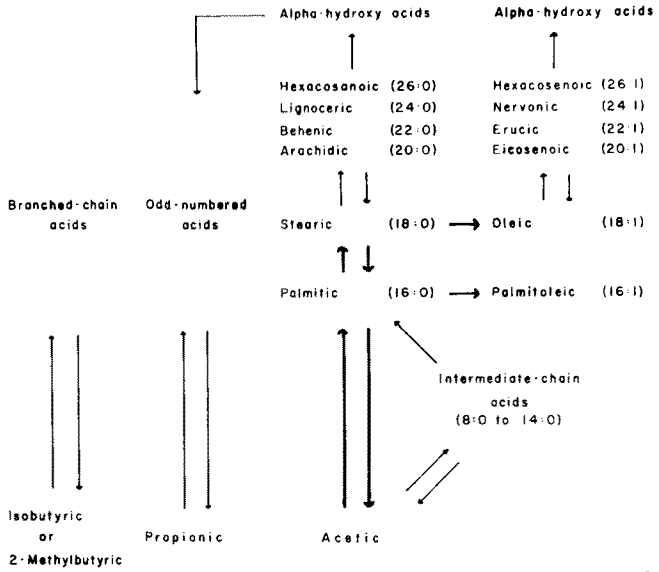

FIG. 5. Some biosynthetic pathways leading to saturated and monounsaturated fatty acids in animal tissues.

can of course also occur within the body and may have an important bearing on the fatty acid composition of tissue lipids. Some fatty acids can arise from either endogenous or exogenous sources while others, of which linoleic acid is the most notable example, are not synthesized to any measurable extent in animal tissues and are therefore derived solely from the diet. Still other fatty acids are not synthesized completely in animal tissues but can be formed by transformation of dietary fatty acids. Arachidonic acid, for example, is formed from linoleic by elongation of the carbon chain and insertion of two additional double bonds. The effect of a dietary fatty acid on the composition of tissue lipids depends to some extent on the transformations which it may undergo within the body and on whether or not it can also be synthesized from small molecule precursors by the body tissues. For this reason it is perhaps desirable at this point to consider briefly some of the biosynthetic pathways and interconversions of fatty acids which can occur in animal tissues.

Biosynthesis and Interconversion

Palmitic acid is the main fatty acid formed from small molecule precursors by animal tissue preparations (67,68). It is accompanied by smaller amts of myristic acid and stearic acid and, in mammary gland preparations, intermediate fatty acids ranging from C_8 to C_{12} are also formed to an appreciable extent (69). The major monounsaturated fatty acids in animal tissues, palmitoleic acid and oleic acid, appear to be formed largely from palmitic acid and stearic acid, respectively (70–72). Elongation by the addition of 2-carbon units to existing fatty acids is a common transformation in animal tissues (67). Stearic acid can be formed in this way from palmitic acid (73), and the extra long chain saturated and monounsaturated fatty acids which characterize brain cerebrosides can also be formed by elongation of C_{16} and C_{18} fatty acids (74,75). The α-hydroxy fatty acids and fatty acids with odd numbers of carbon atoms which occur in cerebrosides are readily synthesized by brain tissue, the former by hydroxylation of the corresponding straight-chain acids and the latter by decarboxylation of the α-hydroxy acids (75,76,77).

Odd-numbered fatty acids and branched-chain fatty acids are found in small amts in many animal tissue lipids and these can be synthesized completely from

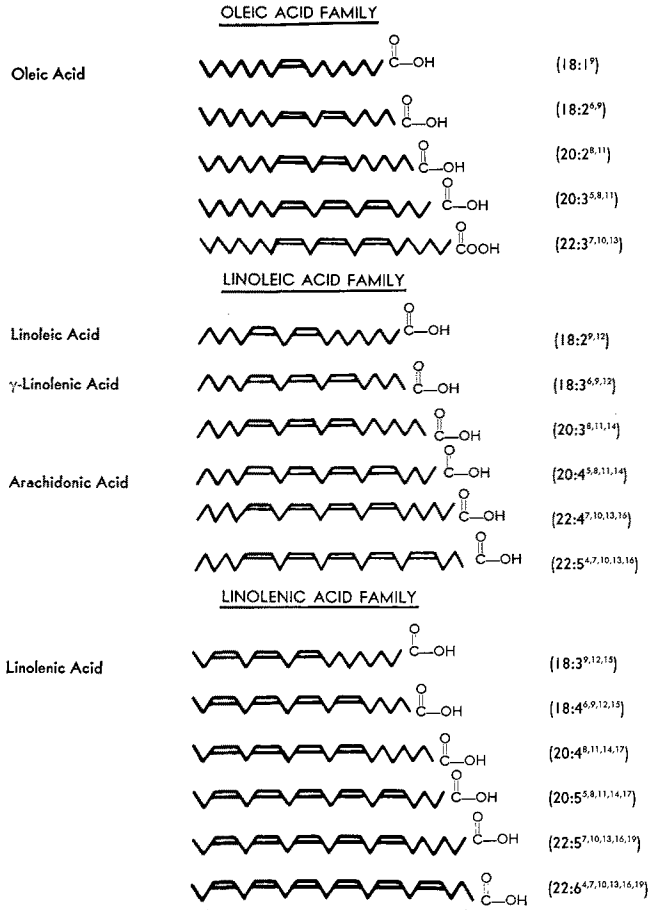

FIG. 6. Families of polyunsaturated fatty acids.

small molecule precursors. Odd-numbered fatty acids are formed when the methyl end of the fatty acid chain is derived from propionic acid (71,78), and branched-chain fatty acids are formed when the methyl end is derived from branched-chain precursors such as isobutyric acid or 2-methylbutyric acid (79). Some of the biosynthetic pathways by which saturated and monounsaturated fatty acids may be formed in animal tissues are summarized in Figure 5.

Another transformation which occurs readily in animal tissues is chain elongation of unsaturated fatty acids with insertion of additional double bonds between the carboxyl group and the existing double bonds. This pathway gives rise to families of polyunsaturated fatty acids (Fig. 6) in which the members of each family have the same configuration at the methyl end of the chain but differ in chain-length and/or total number of double bonds (80,81).

Dietary Fat as a Source

Dietary fat may represent as much as 40 to 50% of the total caloric intake and its fatty acid composition may vary widely depending on the original source and on the changes which result from processing and cooking (82). The potential contribution of dietary fatty acids to the composition of tissue lipids is thus very large indeed. The actual contribution depends on a number of factors and varies considerably from one tissue to another and from one lipid class to another. In tissues and fluids through which the dietary fatty acids pass after absorption from the intestine, a large but rather transient effect may be observed. Chylomicrons, for example, reflect to a considerable extent the composition of dietary fat (83–85) although even at this level dietary fatty acids may be diluted with large amts of endogenous

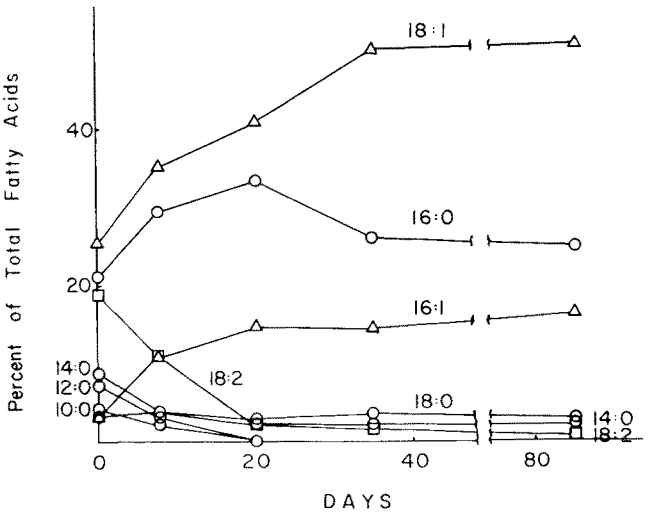

FIG. 7. Changes in fatty acids of adipose tissue in rats weaned to a fat-free diet.

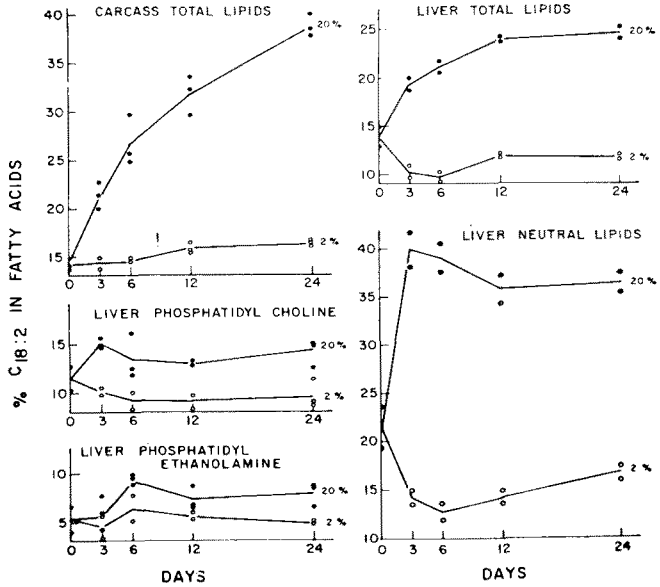

FIG. 8. Changes in levels of linoleic acid in liver and carcass lipids of rats fed diets containing either 2% or 20% corn oil (91).

fatty acids (86). The liver plays an important role in clearing chylomicrons from the blood (87–90) and the fatty acid composition of liver triglycerides is altered rapidly by changes in dietary fat (91). A fairly high proportion of the total fatty acids in storage form in the fat depots may also come from dietary fat but changes in composition of adipose tissue lipids occur rather slowly (6,7). In tissues such as brain, the contribution of dietary fat is usually quite small but even in brain changes due to dietary fat have been reported (92–94).

The fatty acid composition of most body tissue lipid seems to be little affected by day to day variations in the amt and kind of fatty acids in the diet, in spite of the fact that most dietary fats are well absorbed even at high levels of intake (2). This may be explained in part by a dilution effect since the total amt of lipid in the body is in most instances relatively large in comparison to the daily intake of fat. Another factor is the rate of turnover of tissue lipids. Certain lipids in the brain, for example, turn over very slowly if at all (95) and the apparent rapid turnover rate in other tissues deduced from early isotope studies may involve a relatively small fraction of the total lipid. Hirsch has suggested the possibility of two pools of fatty acids in adipose tissue with different rates of turnover, to account for the very long times required to alter the composition of fat depots in humans by dietary fat (7).

There may also be compensatory changes in rates of biosynthesis or of oxidation of fatty acids which tend to minimize changes in tissue fatty acid composition. Fatty acid biosynthesis appears to be regulated in part by the intake of dietary fat, since incorporation of labelled acetate into fatty acids by animal tissues is depressed by increasing the intake of dietary fat (96–98). This does not, however, seem to be a specific effect in the sense that a particular dietary fatty acid inhibits its own synthesis. Rous and Favarger found that incorporation of labelled acetate into palmitic acid by mice was not depressed by feeding palmitic acid and a similar result was obtained with stearic acid (99). Newly ingested fatty acids are oxidized to carbon dioxide more readily than fatty acids which already form part of the tissue lipids (100) and this may be of greater importance than feedback control of fatty acid biosynthesis in maintaining the constancy of tissue fatty acid patterns.

It is commonly found that the fat stores of infants or young animals are more readily altered by diet than those of adults and a number of possible reasons for this have been suggested by Sweeney et al. (101). Growing animals are accumulating new tissue mass, including tissue lipid, whereas the total mass in adults remains approximately constant under normal conditions. The caloric intake of infants is greater in relation to total body weight than that of adults and the fat intake relative to body fat is also greater. Energy expenditure relative to body weight is higher in infants and this may result in more rapid turnover of depot fats which could in turn lead to more rapid changes in the composition of depot fats as a result of changes in dietary lipid.

The specific distribution of fatty acids in different tissue lipid classes is no doubt due in part to selective incorporation of fatty acids into particular classes of lipids and in some cases into particular positions in the lipid molecules. It was noted earlier that phospholipids tend to have higher levels of stearic acid than neutral lipids, and experiments have shown that labelled stearic acid is incorporated preferentially into phospholipids (102,103). The preferential incorporation of labelled linoleic acid into the β-position of lecithin is also in agreement with the observed fatty acid composition of this position (104).

The pattern of incorporation of fatty acids into a particular lipid will be determined to some extent by the mixture of fatty acids available at the site of incorporation. There are, however, limits to the extent to which a tissue lipid can be enriched in a particular fatty acid by dietary means. Tove and Smith were unable to raise the level of oleic acid above about 67% in the fat depots of mice by feeding high levels of oleic acid, glycerol monooleate or olive oil, and suggested that this was due to a resistance to the formation of triolein (105). A possible alternative explanation might be that the dietary oleic acid is diluted with other endogenous fatty acids to an extent that precludes higher levels in the tissue triglyceride. This explanation would not suffice, however, for fatty acids such as stearic acid which seem to have much lower maximum levels in depot fats even when large amts are ingested.

In some cases differences in rates of metabolism

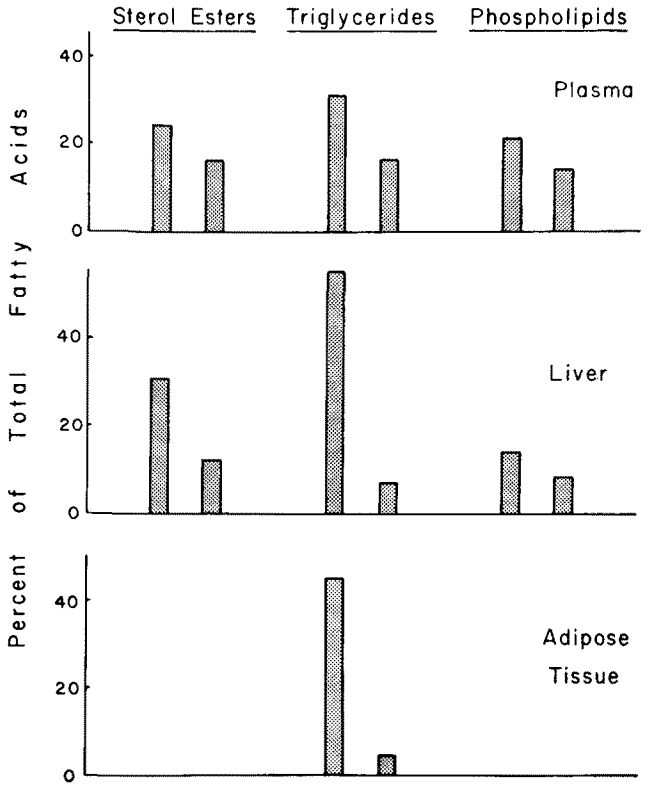

FIG. 9. Linoleic acid levels in lipid classes of plasma, liver and adipose tissue of male rats fed cottonseed oil (left-hand bars) or coconut oil (right-hand bars) at the 10% level for 7 weeks. Safflower oil was used in place of cottonseed oil in the experiment on adipose tissue. The safflower oil contained 75%, the cottonseed oil 54% and the coconut oil 3% linoleate.

or of removal of tissue lipids containing different fatty acids may be more important in determining fatty acid profiles than rate of incorporation of fatty acids into the tissue lipids or rate of entry of particular lipids into the tissue. For example, Swell et al. obtained evidence that cholesteryl linoleate was mobilized more rapidly from rabbit aorta than cholesteryl oleate and suggested that this was mainly responsible for the accumulation of cholesteryl oleate in the aorta of rabbits fed cholesterol (106). Stein and Stein found no differences between palmitic, oleic and linoleic acids in rate of mobilization from rat epididymal fat pads during fasting (107), but Di Giorgio et al. reported that palmitoleic acid was released in greater amts and oleic acid in lesser amts than expected from the tissue lipid composition when adipose tissue was incubated in vitro with epinephrine (97).

Effect of Dietary Fatty Acids

In considering the effect of different types of dietary fatty acids on the fatty acid composition of tissue lipids one must recognize a marked distinction between fatty acids which can be synthesized endogenously and those which are dependent on a dietary source. The former will continue to form part of the tissue lipids whether or not they are present in the diet and their levels may in fact increase in animals transferred to fat-free diets whereas the levels of the latter will almost inevitably fall in the absence of a containing dietary supply. This is illustrated by data of Gellhorn et al. (Fig. 7) which shows changes in adipose tissue fatty acids when weanling rats are placed on a fat-free diet (108). Fatty acids such as linoleic acid, lauric acid and capric acid, which were presumably derived from ingested milk rather than from tissue biosynthesis,

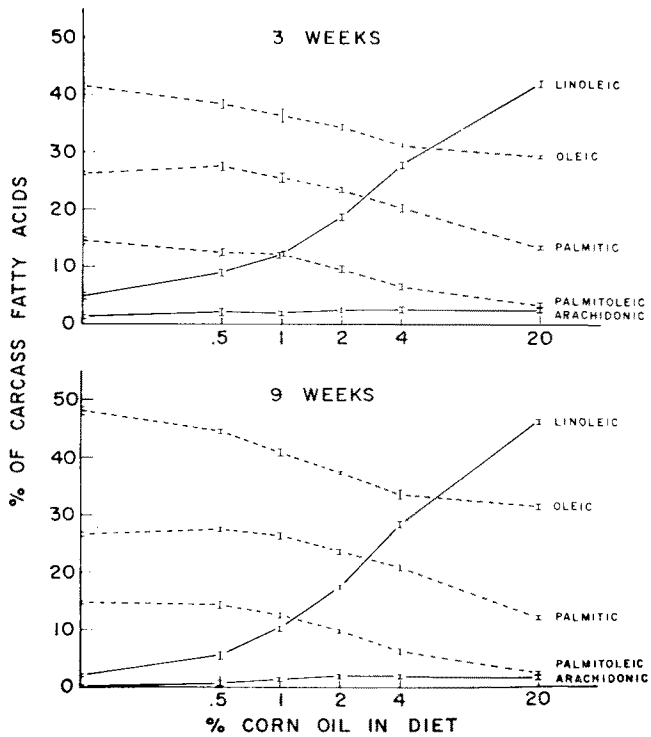

FIG. 10. Levels of different fatty acids in carcass lipids of rats fed diets containing various amts of corn oil for periods of 3 to 9 weeks (91).

decrease rapidly in concn, while palmitic, palmitoleic, stearic and oleic acids either increase in concn or remain at about the same level.

Whereas the tissue levels of linoleic acid decrease rapidly under these conditions, they may show striking increases when the level of linoleic acid in the diet is raised. This is illustrated in Figure 8, taken from a paper by Beare and Kates (91). It is obvious that linoleic acid competes very well with other fatty acids for incorporation into tissue lipids. This figure also illustrates the selectivity with which a fatty acid is incorporated into different lipid classes since the increase in liver neutral lipids is much greater than that in liver phospholipids. It may be noted as well that the level of linoleic acid rises much more quickly in liver neutral lipids than in either liver total lipids or carcass total lipids.

Another example of differences between lipid classes in the ease with which linoleic acid levels may be altered is found in results of Okey et al. (109–111) (Fig. 9). It is seen that the differences in linoleic acid levels between rats fed cottonseed oil or safflower oil and those fed coconut oil are greatest in triglycerides and least in phospholipids, with intermediate values for steryl esters. It is perhaps unwise, however, to generalize on the ease with which fatty acid profiles in different tissue lipid classes can be altered by diet. Hill et al. recently reported greater variations in linoleic acid content of steryl esters and phospholipids of red blood cells than in triglycerides as a result of changes in diet (41).

When the levels of linoleic acid in tissue lipids are varied by altering the dietary intake, there must obviously be compensatory changes in the levels of other fatty acids. The data of Gellhorn et al. (Fig. 7) and of Beare and Kates (Fig. 10) indicate that the levels of all three major fatty acids, palmitic, palmitoleic and oleic are affected. In addition to changes in fatty acid composition due to deposition of linoleic acid itself, changes in other polyunsat-

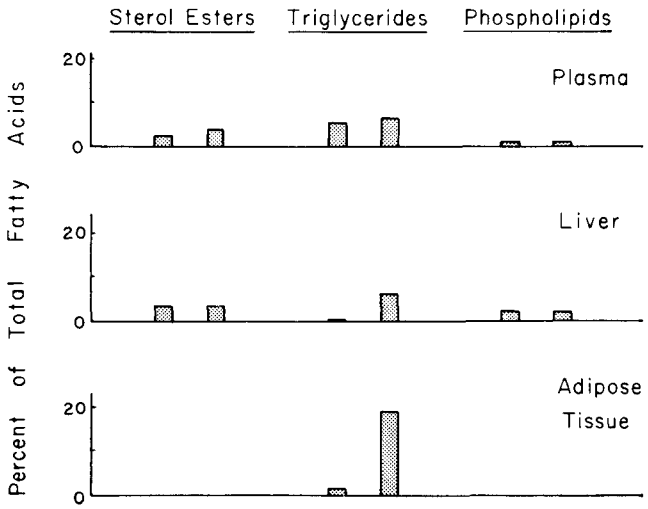

FIG. 11. Levels of fatty acids with chain length less than C_{16} in lipid classes of plasma, liver and adipose tissue of rats fed cottonseed oil or safflower oil (left-hand bars) or coconut oil (right-hand bars) at the 10% level for 7 weeks. The levels in adipose tissue are for lauric acid alone. These results are taken from the same experiments as those shown in Fig. 9. The coconut oil contained 50% laurate and 18% myristate.

urated fatty acids may result from variations in the amt of linoleic acid in the diet. Arachidonic acid is synthesized from linoleic acid under normal circumstances and when linoleic acid is absent from the diet or present in very small quantities, arachidonic acid decreases in amt and is replaced by an eicosatrienoic acid derived from oleic acid (81). An eicosatrienoic acid derived from palmitoleic acid has also been observed in these circumstances (112). The observations of Holman and Mohrhauer suggest that there is competitive inhibition between linolenate, linoleate and oleate for the formation of polyunsaturated fatty acids and that the enzyme-substrate affinities are in the order linolenate > linoleate > oleate (113). This could explain why metabolism of oleate to higher unsaturated fatty acids takes place only when linolenate and linoleate are present in very low concn. The eicosatrienoic acid formed from oleic acid in essential fatty acid deficiency resembles arachidonic acid in its distribution in tissue lipids, being found mainly in phospholipids and steryl esters (18,31). Eicosatrienoic acid and arachidonic acid both appear to be attached mainly to the β-position of glycerophosphatides (114,115).

The essential fatty acids are perhaps the most important group of fatty acids which are derived primarily from the diet. Some information on their contributions to animal tissue lipid classes has been given in the preceding paragraphs and they will not be considered further at this time because they have already been the subject of numerous review articles (116,117). There are, however, various other types of fatty acids which are synthesized to a limited extent or not at all in animal tissues but which may be incorporated into animal tissues from the diet. The next section will deal with experimental studies on some of these fatty acids.

Short-Chain Fatty Acids

Short-chain fatty acids have long been recognized as characteristic components of cow's milk (1,2) and are present to a lesser extent in human milk (118, 119). Milk is therefore an important dietary source of short-chain fatty acids. Coconut oil is also characterized by its high content of short-chain acids, as are the seed oils of a number of other plant families (1,120). The proposed use in edible foods of acetylated monoglycerides containing both long- and short-chain fatty acids in the same molecule introduces another possible dietary source (121–123).

The absorption of short-chain ($< C_{12}$) fatty acids from the intestine differs from that of long-chain fatty acids in that the former are absorbed via the portal system while the latter are transported from the intestine to the blood stream mainly via the lymph (124–126). The fatty acids transported via the portal vein appear to be in the unesterified form (127) while those transported in the lymph are in the form of trigylcerides, phospholipids or cholesteryl esters. The data available are compatible with the concept that the distribution of absorbed fatty acids between the portal capillaries and the intestinal lymph is largely determined by the extent to which newly absorbed fatty acids are incorporated into triglycerides, phospholipids, etc., in the intestinal mucosa (128,129).

Short-chain fatty acids are oxidized at a faster rate in the animal body than long-chain fatty acids (130) and the feeding of short-chain fatty acids does not lead to any appreciable deposition in tissue lipids (131–136) although capric acid has been reported to make up 4.5% of the total acids in adipose tissue of suckling rats (108) and up to 7% of capric (10:0) and lauric (12:0) acids was found in the skin lipids of rats fed mixed triglycerides containing fatty acids of intermediate chain length (137). Reiser et al. (98) reported that dietary lauric acid was not deposited in rat liver lipids but that it led to greater deposition of myristic acid (14:0) than myristic acid itself. Figure 11 shows the levels of fatty acids with less than 16 carbons in various tissue lipid classes of rats fed coconut oil as compared to rats fed cottonseed or safflower oil (109–111). The greatest deposition occurred in adipose tissue of rats fed coconut oil. There was also some deposition in liver triglycerides but otherwise the levels were relatively unaffected. It is obvious that dietary short-chain fatty acids are deposited in tissue lipids less readily than linoleic acid (cf Fig. 9).

Extra-Long Chain ($>C_{18}$) Fatty Acids

Saturated fatty acids with chain-length greater than C_{14} are poorly absorbed when fed as the sole source of fat in the diet (138,139) but absorption is enhanced when they are fed together with unsaturated fatty acids or as mixed triglycerides (140,141). Fields and Gatt observed that 1-^{14}C-labelled lignoceric acid (24:0) was absorbed to the extent of 15–30% when given to rats by stomach tube and its distribution in lymph lipids was similar to that of other long-chain fatty acids (142). Gatt also studied the metabolism of labelled lignoceric acid following intravenous injection (143,144). The radioactivity was cleared rapidly from the blood but its rate of incorporation into liver lipids and the turnover of these lipids was much slower than that of palmitic acid (145). A considerable portion of the radioactivity in liver lipids was found in sphingolipids and, in contrast to palmitic acid, a large proportion of the labelled lignoceric acid remained for hours in the free form. Tests in vitro showed that lignoceric acid did not form a soluble complex with albumin and in this respect it also differs from shorter-chain fatty acids (146). These studies provide evidence of marked differences in the metabolism of saturated fatty acids of different chain length.

The long-chain monounsaturated fatty acids, eicosenoic acid (20:1) and erucic acid (22:1) (Fig. 12) are of interest as major components of rapeseed oil (147). This oil is one of the main seed oils of the world in terms of total quantity produced (148) and is one of the few oils which can be produced economically from a plant growing in northern climates. Rapeseed oil is widely used for food purposes in the Far East and also in Scandinavia and in other countries of northern Europe (149).

Rapeseed oil is more slowly absorbed and metabolized than most other common oils, presumably because of its high erucic acid content (150). However, digestibility studies have shown that long-chain monounsaturated fatty acids are more readily absorbed by rats than their saturated counterparts (138, 151). Rats and dogs fed rapeseed oil deposit erucic acid in tissue lipids (152,153) and when eicosenoic acid and erucic acid were fed to rats as methyl esters at a level of 5% of the diet, they were deposited in body fat to the extent of 28% and 12%, respectively (151). Most of the erucic acid is incorporated into neutral lipids and relatively little is found in plasma and tissue phospholipids (153,154). When rapeseed oil or erucic acid is fed to rats, the conen of adrenal cholesteryl esters increases about fourfold and 35 to 50% of the cholesterol is esterified with erucic acid (154) (Fig. 13). A probable explanation is that the ester accumulates because it is metabolized more slowly than other steryl esters. It is interesting, however, that adrenal cholesteryl esters normally contain fairly large amts of a C_{22} tetraenoic acid (39, 155) whose level decreases as the level of erucic acid increases. It has also been found that a C_{22} trienoic acid accumulates in adrenal cholesteryl esters of rats with essential fatty acid deficiency (156). In rats fed rapeseed oil or erucic acid the intestinal wall becomes thickened and the intestinal mucosa takes on a milky appearance due to the presence of emulsified fat, mainly in the form of triglyceride (157). This may be due to slow removal of glycerides containing erucic acid from the intestinal mucosa.

Studies with ^{14}C-labelled erucic acid and nervonic acid administered to rats by mouth showed that they were deposited in liver lipids to a lesser extent and converted to respiratory CO_2 more slowly than labelled palmitic acid (158). Further experiments in which the liver lipids were separated into different lipid classes showed that erucic acid labelled neutral lipids preferentially while nervonic acid contributed more label to phospholipids (Fig. 14). Both erucic acid and nervonic acid resembled lignoceric acid in that radioactivity was present in the liver in large amts as free fatty acid following their intravenous injection, but the radioactivity did not persist in this form as long as in the case of lignoceric acid. In vitro experiments showed that nervonic acid, like lignoceric acid, failed to form a complex with serum albumin. Erucic acid complexed with albumin, but less readily than did palmitic acid (157).

Long-chain polyunsaturated fatty acids are major components of the depot fats of fish and other marine life, and as such they may be ingested in considerable amts by land animals, including man. The fat depots of land animals normally contain no more than trace amts of these fatty acids (1) but long-chain polyunsaturated fatty acids accumulated in the three major lipid classes of the serum of humans fed menhaden oil (5). Birds and mammals which subsist largely on fish have increased amts of these fatty

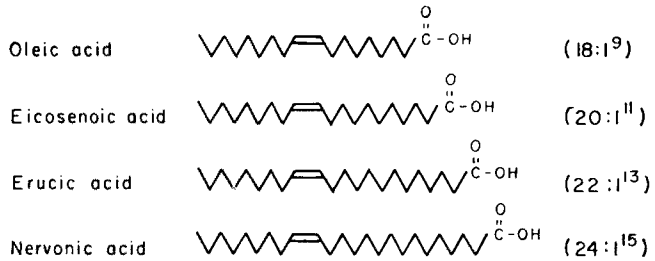

FIG. 12. Structures of some monounsaturated fatty acids.

acids in their tissue lipids (1,160) and it seems likely that they are derived from the diet. In view of the time required to produce changes in the fatty acid composition of depot fats in species such as man, it may be necessary to feed fish oils consistently over long periods in order to demonstrate their deposition in human depot fats.

Reiser et al have provided evidence that the long-chain polyunsaturated fatty acids of fish are of dietary origin since they are readily depleted from the tissue lipids when fish are placed on a fat-free diet (161) (Fig. 15). Reiser et al. also studied the conditions necessary for conversion of linoleic acid and linolenic acid to longer-chain polyunsaturated fatty acids in fish. If linoleic or linolenic were fed at levels of 5% or more there was little conversion, but appreciable conversion was observed if the linoleic and linolenic acids were fed at the 1% level or if they were deposited in tissues previously depleted of C_{20} and C_{22} acids and the fish again depleted. The reasons for larger amts of polyunsaturated long-chain fatty acids in marine fish compared to fresh-water fish have been discussed by Farkas and Herodek (162). They consider that these fatty acids are synthesized in the first instance mainly by crustaceans on which the fish feed and evidence is provided that crustacean lipids become more unsaturated as the temp of the water decreases.

Trans Unsaturated Fatty Acids

Trans unsaturated fatty acids are important components of dietary fats in terms of quantity since they are formed during commercial hydrogenation of fats and may constitute 20 to 40% of the total fatty acids of margarines and shortenings (163). The

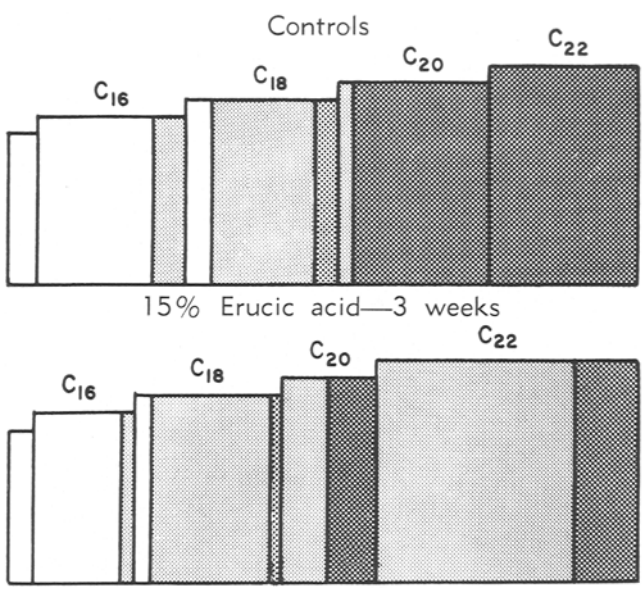

FIG. 13. Fatty acid profiles of adrenal cholesteryl esters of control rats and rats fed a diet containing 15% erucic acid for 3 weeks.

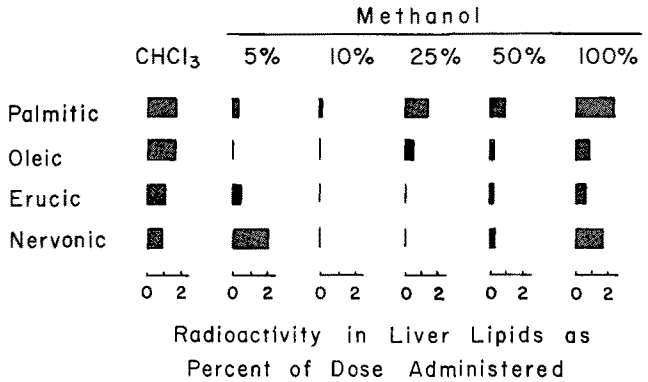

FIG. 14. Distribution in rat liver lipids of radioactivity from different labelled fatty acids administered orally 6 hr before the animals were killed. The different columns represent fractions separated by chromatography on acid-treated Florisil (159).

body fats of ruminants contain *trans* fatty acids which are thought to be derived from hydrogenation of dietary fatty acids by rumen bacteria (164). Appreciable amts of *trans* fatty acids have also been demonstrated in the lipids of human tissue (165) while rats fed a diet containing margarine deposited large amts of *trans* acids in their carcass fat (166). The carcass fat of new-born rats was found to contain less than 0.5% of *trans* fatty acids although the carcass fat of the mothers contained 24 to 29% of *trans* acids as a result of feeding hydrogenated margarine (167). This indicates that *trans* fatty acids are not transferred from mother to young prior to birth. The occurrence of *trans* fatty acids has recently been reviewed and some of their nutritional aspects discussed by Kaufmann and Mankel (168).

Trans double bonds produce little deformation in a carbon chain, in contrast to *cis* double bonds and in some respects the properties of a *trans* fatty acid are intermediate between those of its *cis* isomer and the corresponding saturated fatty acid. Elaidic acid, for example, is a solid melting at 44C, while oleic acid, its *cis* isomer, is liquid at room temp and stearic acid, the corresponding saturated fatty acid, melts at 70C. Since *trans* fatty acids differ in shape and in physical properties from their *cis* isomers, it is reasonable to expect that they should also differ in biological properties and that these differences should be reflected to some extent in the properties of lipids which contain *trans* as opposed to *cis* fatty acids.

Elaidic acid was used before the advent of radioactive fatty acids as a marker to study the fate and distribution of dietary fat (169,170). The feeding of elaidin, the triglyceride of elaidic acid, to rats, led to rapid incorporation of elaidic acid into the phospholipids, replacing 25 to 30% of the natural fatty acids, particularly the saturated ones. Elaidic acid appeared in liver and muscle and, to a lesser extent in brain phospholipids. In the intestinal tract elaidic acid replaced as much as half of the phospholipid fatty acids, with saturated and unsaturated acids being replaced to an equal extent. These studies by Sinclair were later extended by Kohl, who showed that elaidic acid was incorporated into triglycerides and was deposited in large amts in the fat depots (171). Raulin et al. analyzed the depot triglycerides of rats fed isomerized peanut oil and found that the *trans* fatty acids were mainly in the α-position of the glyceride molecules (172). Dhopeshwarkar and Mead (173) found that methyl elaidate was deposited unchanged in guinea pig tissues. Coots (103) has recently reported that ^{14}C-labelled elaidic acid is absorbed, transported in the lymph and converted to respiratory CO_2 in the rat in much the same way as labelled oleic and palmitic acids.

Privett and Blank (18) fed the *trans,trans* isomer of linoleic acid (linoelaidic acid) to rats deficient in essential fatty acids and found that it was desposited in the steryl esters, triglycerides and phospholipids of all tissues examined. They reported that it was not converted to longer-chain polyunsaturated fatty acids, although it inhibited accumulation of the eicosatrienoic acid which is characteristic of essential fatty acid deficiency. *Cis*-9-*trans*-12-octadecadienoic acid also inhibited accumulation of eicosatrienoic acid but was itself converted to longer-chain polyunsaturated acids, probably by means of steps similar to those occurring in the metabolism of the natural *cis* isomer (174). Knipprath and Mead (175) have recently reported that *trans,trans* octadecadienoic acid is also converted to longer-chain fatty acids by the rat, in contrast to the results of Privett and Blank. *Trans* isomers of linoleic acid are oxidized to respiratory CO_2 to a greater extent than the natural *cis* isomer but their digestion and absorption in the rat follows a pattern similar to that of natural linoleic acid (176).

Neither the *trans* isomers nor their metabolites are able to prevent the symptoms of essential fatty acid deficiency (177,178) and evidence has been presented that *trans* fatty acids actually accentuate the symptoms of essential fatty acid deficiency (116,179). Although this evidence has been questioned (180), it does seem that the *trans* isomers inhibit accumulation of longer-chain polyunsaturated acids derived from *cis* isomers (18). Long-chain fatty acids derived from the *trans* isomers are found primarily in phospholipids and steryl esters and in this respect they are similar to arachidonic acid which is incorporated into triglycerides only to a limited extent.

Conjugated Fatty Acids

Conjugated fatty acids have double bonds alternating with single bonds rather than being separated by a methylene group as in most naturally-occurring fatty acids. Conjugated fatty acids are formed during hydrogenation of fats and hence are present in typical American margarines and shortenings (163). Christensen et al. reported that conjugated dienoic fatty acids accumulated to a maximum of 2% in depot fats of rats fed on a diet containing 28% of hydrogenated peanut oil (181). Aaes-Jorgensen fed individual conjugated acids containing both *cis* and *trans* double bonds and found that they accumulated in depot fats and heart lipids (182). These acids had no essential fatty acid activity nor did they appear to accentuate essential fatty acid deficiency.

Alpha-eleostearic acid is a naturally-occurring conjugated fatty acid (9-*cis*,11-*trans*,13-*trans*-octadecatrienoic acid) which makes up more than 70% of the total fatty acids of tung oil. When eleostearic acid is fed to rats or chickens it is largely converted into a fatty acid with only two conjugated double bonds as indicated by UV absorption studies (183–185). Rats fed a diet containing 30% tung oil continued to grow and were reasonably healthy over a period of many months. At autopsy, their adipose tissues were found to contain 50% of the conjugated dienoic acid (186).

Acetylenic Fatty Acids

Fatty acids containing a triple bond are rare in naturally-occurring fats and oils but a few, such as tariric acid (6-stearolic acid), ximenic acid and

ximenynic acid are found as components of certain seed oils (1,2,187,188). Little is known of their metabolism and fate in animals. Bernhard and Gloor (189) fed deuterated stearolic, behenolic and undecynoic acids to dogs and reported that they were oxidized at the triple bond since labelled azelaic acid was found in the urine. On the other hand, Dear and Pattison (190) found no evidence for oxidation or cleavage of the acetylenic link in toxicity studies with acetylenic fatty acids fluorinated in the ω-position.

Branched-Chain Fatty Acids

Branched-chain acids are normal constituents of many lipids and are especially characteristic of bacterial lipids (191–195). Their occurrence in mammalian tissues has been studied especially by Shorland et al. These workers also investigated the metabolism in rats of naturally-occurring C_{15} and C_{17} anteiso-acids (196,197). Small amts were stored in carcass lipids and interconversion of the two acids was demonstrated. Their metabolic turnover appeared to be considerably slower than that of normal fatty acids.

A number of synthetic branched-chain acids of unnatural structure have been used as investigative tools of fat metabolism (194). Bergstrom et al. (198) studied the metabolism of 2,2-dimethylstearic acid and found that it was readily absorbed by the rat and transported in the lymph in much the same way as ordinary long-chain fatty acids. It was also incorporated into triglycerides and phospholipids after it had been administered as the free acid. However, it differed from ordinary fatty acids in two main respects. Glyceride ester bonds of the branched-chain acid were almost resistant to pancreatic lipase and the normal oxidation pathway of β-oxidation was apparently also hindered by the methyl groups and ω-oxidation occurred instead, giving rise to dimethyl adipic acid which was excreted in the urine. Tryding and Westöö also found evidence for ω-oxidation of 2,2-dimethylnonadecanoic acid (199) and to a small extent of 2-methyl stearic acid (200), but 2,2,17,17-tetramethylstearic acid was inert to both β- and ω-oxidation and was excreted in the feces largely as the unchanged acid (201). Stern and Treadwell (202) found that cholesteryl esters of branched-chain acids were less readily hydrolyzed by cholesteryl esterases than those of straight-chain acids.

Goodman and Steinberg (203) studied the fate of 3,3-dimethyl-14-phenylmyristic acid. They used a 3-substituted acid because they believed that the methyl groups would cause less steric hindrance but would still prevent β-oxidation. The phenyl substituent was used to increase the solubility of the fatty acid salts relative to a fatty acid substituted in the ω-position with methyl groups. The experimental results justified the assumption that this fatty acid was not readily degraded by β- or ω-oxidation. The fatty acid was bound to albumin at least as tightly as palmitic acid, but relatively large amts of it in the plasma did not appear to interfere significantly with palmitate transport to sites of oxidation. ω-Oxidation of the ordinary straight-chain fatty acids such as stearic, oleic and palmitic has recently been demonstrated in vitro by Preiss and Bloch (204), using aged rat liver microsomes, but the biological significance of this is not clear.

Fatty acids containing a cyclopropane or cyclopropene ring, which are present in the lipids of certain kinds of bacteria (205), constitute a special type of branched-chain fatty acid. Cottonseed oil

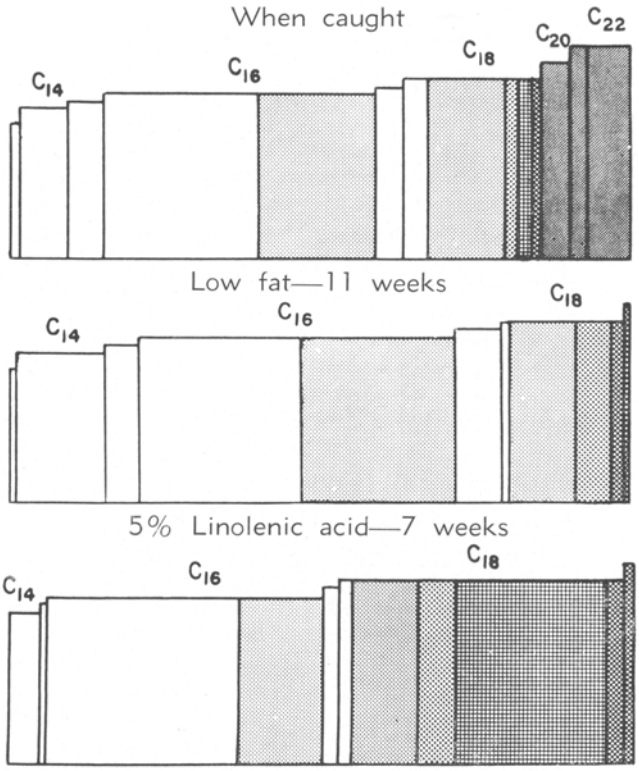

FIG. 15. Fatty acid profiles of a salt-water fish on its natural diet and after periods of being fed a fat-free diet or a diet containing 5% linolenic acid.

and *Sterculia foetida* seeds also contain sterculic and malvalic acids, which are characterized by a cyclopropene ring. The feeding of these seed oils leads to higher concns of stearic acid in the tissue lipids of chickens (206) and similar observations have been made in pigs and in fish fed cottonseed oil (207,208). It has been suggested that the cyclopropene fatty acids either promote the complete saturation of dietary linoleic acid to stearic acid or that they interfere with the desaturation of endogenously synthesized fatty acids (206). The latter suggestion seems on the whole more plausible (209). The feeding of cyclopropene fatty acids to hens gives rise to a number of undesirable physiological effects such as pink egg yolks and at higher levels, inhibition of sexual development, decreased egg production and embryo mortality in fertile eggs. These effects may be due to reaction of the cyclopropene ring with sulphydryl groups in the body proteins (210).

Oxygenated Fatty Acids

Epoxy acids are formed in the catalytic oxidation of unsaturated fatty acids and they also occur naturally in a number of seed oils. Chalvardjian et al. (211) carried out feeding experiments in rats with the seed oil of *Vernonia anthelmintica* which contains about 70% of vernolic acid (12,13-epoxyoleic acid) (212) and demonstrated the presence of epoxy acid in the lipids of liver, kidney, heart, and adipose tissue. In adult rats fed the *Vernonia* seed oil for 10 to 15 days as 10% of the diet, the epoxy fatty acid made up about 2% of the total fatty acids of adipose tissue while the levels in other tissues were below 1%. Weanling rats fed the oil for 28 days had 14% epoxy acid in their epidydimal fat. Infrared spectra gave no evidence of conversion of the epoxy acid to hydroxy acid. Growth was not depressed by this oil and no adverse effects on the gross or microscopic anatomy of the animals were observed. Epoxyoleate

did not prevent symptoms of essential fatty acid deficiency nor did it appear to act as a metabolic antagonist of linoleic acid (213).

Included among the oxidation products of fatty acids are lipid peroxides (214–217). These can be demonstrated in tissue lipids after ingestion of highly unsaturated fatty acids, particularly in animals dificient in the tocopherols which serve as natural antioxidants (218). The lipid peroxides are probably formed within the body since Mead reported that no peroxide was found in the thoracic duct lymph of rats fed lipid peroxides (219) (see also 220,221).

Hydroxy acids form a rather large proportion of the total fatty acids of thermally oxidized oils (222) and such oils cause growth depression and other toxic symptoms when fed to experimental animals. Perkins et al. (222) fed oxidized corn oil at the 10% level to weanling rats for 59 days and found 4 to 7% of hydroxy acids in the carcass lipids. Hydroxy acids have been postulated as intermediates in the conversion of saturated acids to monounsaturated acids in animal tissues (70). They also occur naturally in some seed oils (223–225), the best-known example being ricinoleic acid which makes up about 90% of the total fatty acids of castor oil (2,226). Rats fed ricinoleic acid either as the free acid or as the triglyceride at 10% of the diet had levels of 5.5 and 7.5%, respectively, of hydroxy acids in the carcass lipids (227). Ricinoleic acid itself accounts for most of the hydroxy acid deposited but Uchiyama et al. (228) showed that C_{16}, C_{14}, and C_{12} hydroxy acids were also deposited in small amts. Watson and Gordon (229) found that the metabolism of ricinoleic acid was rather similar to that of other long-chain fatty acids but it was converted to a CoA derivative less readily than oleic acid by a system containing intestinal mucosal enzymes.

Dihydroxy acids are rarely found in naturally-occurring fats and oils although they may be present in oxidized oils and dihydroxystearic acid has been reported in castor oil (226). Kaunitz and Johnson (230) fed synthetic 9,10-dihydroxystearic acid to rats at the 5% level and observed some depression in growth. The acid appeared to be well absorbed but analysis by gas-liquid chromatography failed to show any trace in the tissue lipids.

Discussion

Each different fatty acid has its own individual chemical and physical properties which affect to some extent the properties of any lipid molecule of which it forms a part. Thus, although it is convenient to classify lipids on the basis of the nonfatty acid portion of the molecule, it should be borne in mind that the metabolism and function of individual members of a lipid class may be quite different, depending on the nature of their fatty acid substituents. Knowledge of the fatty acid composition of lipid classes and of factors affecting this composition are therefore of basic importance to an understanding of the metabolism and function of tissue lipids.

A specific requirement for particular fatty acids in animal tissue lipids has been recognized since the discovery of the essential fatty acids and many symptoms of essential fatty acid deficiency in animals have been recognized and described (116,117). However, essential fatty acid deficiency appears to be of greater interest from a theoretical than from a practical point of view since most diets contain these fatty acids in adequate amts. Humans resemble other animals in their dietary requirements for essential fatty acids (231,232) and, with the exception of a few reports on infants (233), there is little indication of the occurrence of essential fatty acid deficiency in humans.

The essential nature of linoleic acid and related fatty acids is readily demonstrated because of their dependence on a dietary source. Other common fatty acids may have equally important roles in animal tissues but these are not readily observed because the fatty acids can be synthesized within the body from small molecule precursors. It is conceivable, however, that under some circumstances animals could become deficient in certain of these fatty acids because of a metabolic block in their synthetic pathway and their lack in body tissues could then give rise to deficiency symptoms. This was considered as a possible factor in the etiology of demyelinating diseases since myelin contains extra long-chain fatty acids which are synthesized in the body but which are not normally present in any quantity in human diets (234). Although no experimental evidence was obtained to support this concept (235) it remains a theoretical possibility.

The many symptoms which characterize essential fatty acid deficiency provide good evidence of the requirement in tissue lipids for fatty acids of very specific structure. Lipids are important components of cell membranes (236,237) and their ability to function in membranes probably depends on their having a particular size and shape, which in turn depends in part on the nature of the fatty acid substituents (238). Fatty acid analyses of tissue lipids and attempts to alter fatty acid composition by dietary or other means provides a method of furthering our knowledge of the metabolic and structural role of tissue lipids and extension of this knowledge could have many practical applications. Another approach to the problem of fatty acid specificity in membrane lipids is that of comparative studies in different species as exemplified by the work of Richardson et al. on mitochondrial lipids (239).

The fatty acid analyses which have been discussed in this article deal mainly with whole tissues which contain different types of cells and many types of membranous structures. This makes it difficult to utilize the results for interpretation of the role of lipids in membranes. The relative simplicity of the red cell has encouraged studies on its lipids and on alterations produced by various dietary fats (41,43, 240–242). Such studies may have practical implications in understanding factors involved in maintaining the shape and integrity of red cells.

The greatest stimulus in recent years for research on the relation of diet to tissue fatty acid composition has undoubtedly been the idea that development and progression of atherosclerotic lesions may be affected by the kind of fat in the diet (243–246). The implication of cholesterol in the etiology of atherosclerosis has aroused particular interest in the fatty acid composition and metabolism of cholesteryl esters (32,106,247–249) and has stimulated experimental studies too numerous to mention on the effect of different dietary fats on cholesterol levels in plasma and tissues. Analyses of the fatty acid composition of lipids of arterial walls and blood plasma have also been carried out in a number of laboratories because of their possible bearing on the problem of atherosclerosis (250–252). The demonstrated ability of polyunsaturated fatty acids to lower plasma cholesterol levels in humans has led to suggestions of raising the dietary level of polyunsaturated fats, and several different research groups have been investi-

gating the possible implications of this, particularly in respect to lipid peroxidation and increased requirement for antioxidants such as vitamin E (218). This is another area in which knowledge of tissue lipid composition and the ways in which it is affected by diet may have practical applications.

With the exception of the essential fatty acids, no absolute need for an exogenous source of fatty acids has been demonstrated but dietary fats seem to have beneficial effects in animals (253) and there is some evidence that the ratio of saturated to monounsaturated fatty acids in dietary fat may also have significance (254). The use of high-fat rations for poultry and livestock in recent years accentuates the need for additional studies on the effects of different combinations of dietary fats. The role of fats in human diets also warrants further investigation to provide a background of information for assessing their nutritional or therapeutic value and to increase our understanding of the significance of alterations which may occur during processing and cooking. This review may serve to emphasize the usefulness of such studies and give an indication of the potential for further research.

REFERENCES

1. Hilditch, T. P., and P. N. Williams "The Chemical Constitution of Natural Fats," 4th ed., Chapman & Hall, London, 1964.
2. Deuel, H. J., Jr., "The Lipids," Vols. I to III, Interscience Publishers, Inc., New York, 1951, 1955, 1957.
3. Lovern, J. A., Biochem. Soc. Symposia (Cambridge, England) *6*, 49–62 (1951).
4. Shorland, F. B., "Comparative Biochemistry," Vol. 3, ed. M. Florkin and H. S. Mason, Academic Press, New York, 1962. pp. 1–102.
5. Ahrens, E. H., Jr., W. Insull, J. Hirsch, W. Stoffel, M. L. Peterson, J. W. Farquhar, T. Miller and H. J. Thomasson, Lancet *i*, 115–119 (1959).
6. Hirsch, J., J. W. Farquhar, E. H. Ahrens, Jr., M. L. Peterson and W. Stoffel, Am. J. Clin. Nutr. *8*, 499–511 (1960).
7. Hirsch, J., in "Adipose Tissue as an Organ," ed. L. W. Kinsell, Charles C. Thomas, Springfield, Ill., 1962, pp. 79–125.
8. Kingsbury, K. J., D. M. Morgan and T. D. Heyes, Biochem. J. *90*, 140–147 (1964).
9. Krut, L. H., and B. Bronte-Stewart, J. Lipid Res. *5*, 343–351 (1964).
10. Jangaard, P. M., R. G. Ackman and R. D. Burgher, Can. J. Biochem. Physiol. *41*, 2543–2546 (1963).
11. Erwin, E. S., W. Sterner and G. J. Marco, JAOCS *40*, 344–347 (1963).
12. Tove, S. B., and R. D. Mochrie, J. Dairy Sci. *46*, 686–689 (1963).
13. Wood, R. D., M. C. Bell, R. B. Grainger and R. A. Teekell, J. Nutr. *79*, 62–68 (1963).
14. Ward, P. F. V., T. W. Scott and R. M. C. Dawson, Biochem. J. *92*, 60–68 (1964).
15. Shorland, F. B., Nature *170*, 924–925 (1952).
16. Shorland, F. B., in "Progress in the Chemistry of Fats and Other Lipids," Vol. 3, ed. R. T. Holman, W. O. Lundberg and T. Malkin, Pergamon Press, New York, 1955, pp. 276–325.
17. Hilditch, T. P., Nature *170*, 925–926 (1952).
18. Privett, O. S., and M. L. Blank, JAOCS *41*, 292–297 (1964).
19. Hallgren, B., S. Stenhagen, A. Svanborg and L. Svennerholm, J. Clin. Invest. *39*, 1424–1434 (1960).
20. Dittmer, J. C., and D. J. Hanahan, J. Biol. Chem. 234, 1976–1982 (1959).
21. Swell, L., M. D. Law, P. E. Schools, Jr., and C. R. Treadwell, J. Nutr. *75*, 181–191 (1961).
22. Morin, R. J., S. Bernick, J. F. Mead and R. B. Alfin-Slater, J. Lipid Res. *3*, 432–438 (1962).
23. Leat, W. M. F., Biochem. J. *89*, 44–51 (1963).
24. Tinoco, J., A. Shannon and R. L. Lyman, J. Lipid Res *5*, 57–62 (1964).
25. Brockerhoff, H., Arch. Biochem. Biophys. *93*, 641–645 (1961).
26. Getz, G. S., W. Bartley, F. Stirpe, B. M. Notton and A. Renshaw, Biochem. J. *80*, 176–181 (1961).
27. MacFarlane, M. G., Biochem. J. *78*, 44–51 (1961).
28. Nelson, G. J., J. Lipid Res. *3*, 256–262 (1962).
29. Nelson, G. J., J. Lipid Res. *3*, 71–79 (1962).
30. Sweeley, C. C., J. Lipid Res. *4*, 402–406 (1963).
31. Leat, W. M. F., Biochem. J. *91*, 437–443 (1964).
32. Swell, L., H. Field, Jr., and C. R. Treadwell, Proc. Soc. Exp. Biol. Med. *104*, 325–328 (1960).
33. O'Brien, J. S., D. L. Fillerup and J. F. Mead, J. Lipid Res. *5*, 329–338 (1964).
34. O'Brien, J. S., and G. Rouser, J. Lipid Res. *5*, 339–342 (1964).
35. Rouser, G., This Symposium.
36. Gray, G. M., and M. G. MacFarlane, Biochem. J. *81*, 480–488 (1961).
37. Morgan, T. E., D. O. Tinker and D. J. Hanahan, Arch. Biochem. Biophys. *103*, 54–65 (1963).
38. Shuster, C. Y., J. R. Froines and H. S. Olcott, JAOCS *41*, 36–41 (1964).
39. Chang, T–C. L., and C. C. Sweeley, Biochemistry *2*, 592–604 (1963).
40. Riley, C., Biochem. J. *87*, 500–507 (1963).
41. Hill, J. G., A. Kuksis and J. M. R. Beveridge, JAOCS *41*, 393–397 (1964).
42. Ways, P., and D. J. Hanahan, J. Lipid Res. *5*, 318–328 (1964).
43. Farquhar, J. W., and E. H. Ahrens, Jr., J. Clin. Invest. *42*, 675–685 (1963).
44. Kerr, S. E., and W. W. C., Read, Biochim. Biophys. Acta. *70*, 477–478 (1963).
45. Sambasivarao, K., and R. H. McCluer, J. Lipid Res. *5*, 103–108 (1964).
46. Veerkamp, J. H., I. Mulder and L. L. M. van Deenen, Biochim. Biophys. Acta *57*, 299–309 (1962).
47. Getz, G. S., and W. Bartley, Biochem. J. *78*, 307–312 (1961).
48. Savary, P., J. Flanzy and P. Desnuelle, Biochim. Biophys. Acta *24*, 414–423 (1957).
49. Mattson, F. H., and E. S. Lutton, J. Biol. Chem. *233*, 868–871 (1958).
50. Reiser, R., and H. G. R. Reddy, JAOCS *36*, 97–99 (1959).
51. Brockerhoff, H., and R. J. Hoyle, Arch. Biochem. Biophys. *102*, 452–455 (1963).
52. Mattson, F. H., R. A. Volpenhein and E. S. Lutton, J. Lipid Res. *5*, 363–365 (1964).
53. Tattrie, N. H., J. Lipid Res. *1*, 60–65 (1959).
54. Hanahan, D. J., H. Brockerhoff and E. J. Barron, J. Biol. Chem. *235*, 1917–1923 (1960).
55. Kennedy, E. P., Fed. Proc. *20*, 934–940 (1961).
56. Brockerhoff, H., R. G. Ackman and R. J. Hoyle, Arch. Biochem. Biophys. *100*, 9–12 (1963).
57. Lands, W. E. M., and P. Hart, J. Lipid Res. *5*, 81–87 (1964).
58. Johnston, J. M., in "Advances in Lipid Research," Vol. I, ed. R. Paoletti and D. Kritchevsky, Academic Press, New York, 1963, pp. 105–131.
59. Hofmann, A. F., and B. Bergström, J. Clin. Invest. *43*, 247–257 (1964).
60. Brockerhoff, H., R. J. Hoyle and K. Ronald, J. Biol. Chem. *239*, 735–739 (1964).
61. Privett, O. S., and M. L. Blank, JAOCS *40*, 70–75 (1963).
62. de Vries, B., JAOCS *41*, 403–406 (1964).
63. Subbaram, M. R., and C. G. Youngs, JAOCS *41*, 445–448 (1964).
64. Kuksis, A., M. J. McCarthy and J. M. R. Beveridge, JAOCS *41*, 201–205 (1964).
65. Collins, F. D., Biochem. J. *88*, 319–324 (1963).
66. Renkonen, O., JAOCS *42*, 298–304 (1965).
67. Wakil, S. J., Ann. Rev. Biochem. *31*, 369–406 (1962).
68. Harlan, W. S., Jr., and S. J. Wakil, J. Biol. Chem. *238*, 3216–3223 (1963).
69. Dils, R., and G. Popjak, Biochem. J. *83*, 41–51 (1962).
70. Marsh, J. B., and A. T., James, Biochim. Biophys. Acta *60*, 320–328 (1962).
71. Mead, J. F., Ann. Rev. Biochem. *32*, 241–268 (1963).
72. Erwin, J., and K. Bloch, Science *143*, 1006–1012 (1964).
73. Mead, J. F., Am. J. Clin. Nutr. *6*, 652–655 (1958).
74. Kishimoto, Y, and N. S. Radin, J. Lipid Res. *4*, 444–447 (1963).
75. Hajra, A. K., and N. S. Radin, J. Lipid Res. *4*, 448–453 (1963).
76. Hajra, A. K., and N. S. Radin, J. Lipid Res. *4*, 270–278 (1963).
77. Levis, G. M., and J. F. Mead, J. Biol. Chem. *239*, 77–80 (1964).
78. Hajra, A. K., and N. S. Radin, J. Lipid Res. *3*, 327–332 (1962).
79. Horning, M. G., D. B. Martin, A. Karmen and P. R. Vagelos, J. Biol. Chem. *236*, 669–672 (1961).
80. Klenk, E., Experientia *17*, 199–204 (1961).
81. Mead, J. F., Fed. Proc. *20*, 952–955 (1961).
82. Brown, J. B., Nutr. Rev. *17*, 321–325 (1959).
83. Bragdon, J. H., and A. Karmen, J. Lipid Res. *1*, 167–170 (1960).
84. Kuo, P. T., A. F. Whereat, D. R. Bassett and E. Staple, Circulation *24*, 213–222 (1961).
85. Savary, P., M. J. Constantin and P. Desnuelle, Biochim. Biophys. Acta *48*, 562–571 (1961).
86. Karmen, A., M. Whyte and D. S. Goodman, J. Lipid Res. *4*, 312–321 (1963).
87. Bragdon, J. H., and R. S. Gordon, J. Clin. Invest. *37*, 574–578 (1958).
88. Stein, Y., and B. Shapiro, J. Lipid Res. *1*, 326–331 (1960).
89. Belfrage, P., B. Borgström and T. Olivecrona, Acta Physiol. Scand. *58*, 111–123 (1963).
90. Robinson, D. S., in "Advances in Lipid Research," Vol. I, ed. R. Paoletti and D. Kritchevsky, Academic Press. New York, 1963, pp. 133–182.
91. Beare, J. L., and M. Kates, Can. J. Biochem., *42*, 1477–1486 (1964).
92. Machlin, L. J., G. J. Marco and R. S. Gordon, JAOCS *39*, 229–232 (1962).
93. Century, B., L. A. Witting, C. C. Harvey and M. K. Horwitt, Am. J. Clin. Nutr. *13*, 363–368 (1963).
94. Mohrhauer, H., and R. T. Holman, J. Neurochem. *10*, 523–530 (1963).
95. Davison, A. N., and J. Dobbing, Nature *191*, 844–848 (1961).
96. Hill, R., J. M. Linazasoro, F. Chevallier and I. L. Chaikoff, J. Biol. Chem. *233*, 305–310 (1958).
97. DiGiorgio, J., R. A. Bonanno and C. M. Hegsted, J. Nutr. *78*, 384–392 (1962).
98. Reiser, R., M. C. Williams, M. F. Sorrels and N. L. Murty, Arch. Biochem. Biophys. *102*, 276–285 (1963).
99. Rous, S., and P. Favarger, Helv. Physiol. Pharmacol. Acta *21*, 125–130 (1963).
100. Volk, M. E., R. H. Millington and S. Weinhouse, J. Biol. Chem. *195*, 493–501 (1952).
101. Sweeney, M. J., J. N. Etteldorf, L. J. Throop, D. L. Timma and E. L. Wrenn, J. Clin. Invest. *42*, 1–9 (1963).
102. Dittmer, J. C., and D. J. Hanahan, J. Biol. Chem. *234*, 1983–1989 (1959).
103. Coots, R. H., J. Lipid Res. *5*, 468–472 (1964).
104. Stein, O., and Y. Stein, Biochim. Biophys. Acta *70*, 517–530 (1963).
105. Tove, S. B., and F. H. Smith, J. Nutr. *71*, 264–272 (1960).
106. Swell, L., M. D. Law and C. R. Treadwell, J. Nutr. *81*, 263–270 (1963).
107. Stein, Y., and O. Stein, Biochim. Biophys. Acta *60*, 58–71 (1962).
108. Gellhorn, A., W. Benjamin and M. Wagner, J. Lipid Res. *3*, 314–319 (1962).
109. Lis, E. W., and R. Okey, J. Nutr. *73*, 117–125 (1961).
110. Okey, R., A. Shannon, J. Tinoco, R. Ostwald and P. Miljanich, J. Nutr. *75*, 51–60 (1961).
111. Ostwald, R., R. Okey, A. Shannon and J. Tinoco, J. Nutr. *76*, 341–352 (1962).
112. Fulco, A. J., and J. F. Mead, J. Biol. Chem. *234*, 1411–1416 (1959).
113. Holman, R. T., and H. Mohrhauer, Acta Chem. Scand. *17*, Suppl. 1, 84–90 (1963).
114. De Tomás, M. E., R. R. Brenner and R. O. Peluffo, Biochim. Biophys. Acta *70*, 472–474 (1963).
115. Leat. W. M. F., Biochem. J. *91*, 444–447 (1964).
116. Aaes-Jorgenson, E., Physiol. Rev. *41*, 1–51 (1961).
117. Sinclair, H. M., in "Lipid Pharmacology," ed. R. Paoletti, Academic Press, New York, 1964, pp. 237–273.

118. Insull, W., Jr., and E. H. Ahrens, Jr., Biochem. J. *72*, 27–33 (1959).
119. Garton, G. A., J. Lipid Res. *4*, 237–254 (1963).
120. Miller, R. W., F. R. Earle, I. A. Wolff and Q. Jones, JAOCS *41*, 279–280 (1964).
121. Alfin-Slater, R. B., R. D. Coleman, R. O. Feuge and A. M. Altschul, JAOCS *35*, 122–127 (1958).
122. Ambrose, A. M., D. J. Robbins and A. J. Cox, Jr., Food Res. *23*, 536–549 (1958).
123. Herting, D. C., S. R. Ames and P. L. Harris, Arch. Biochem. Biophys. *74*, 92–99 (1958).
124. Kiyasu, J. Y., B. Bloom and I. L. Chaikoff, J. Biol. Chem. *199*, 415–419 (1952).
125. Blomstrand, R., Acta Physiol. Scand. *34*, 67–70 (1955).
126. Fernandes, J., J. H. van de Kamer and H. A. Weijers, J. Clin. Invest. *34*, 1026–1036 (1955).
127. Borgström, B., Acta Physiol. Scand. *34*, 71–74 (1955).
128. Havel, R. J., Am. J. Clin. Nutr. *6*, 662–668 (1958).
129. Playoust, M. R., and K. J. Isselbacher, J. Clin. Invest. *43*, 878–885 (1964).
130. Kirschner, S. L., and R. S. Harris, J. Nutr. *73*, 397–402 (1961).
131. Powell, M., J. Biol. Chem. *89*, 547–552 (1930).
132. Longenecker, H. E., J. Biol. Chem. *130*, 167–177 (1939).
133. Shorland, F. B., and P. B. D. De La Mare, J. Agric. Sci. *35*, 33–38 (1945).
134. Weitzel, G., H. Schön, F. Gey and U. Roester, Hoppe-Seylers Z. Physiol. Chem. *303*, 184–195 (1956).
135. Fritz, I. B., Physiol. Rev. *41*, 52–129 (1961).
136. Kaunitz, H., C. A. Slanetz, R. E. Johnson and V. K. Babayan, J. Nutr. *73*, 386–390 (1961).
137. Weitzel, G., H. Schön, F. Gey and H. Kalbe, Hoppe-Seylers Z. Physiol. Chem. *301*, 118–131 (1955).
138. Carroll, K. K., J. Nutr. *64*, 399–410 (1958).
139. Renner, R., and F. W. Hill, J. Nutr. *74*, 259–264 (1961).
140. Mattson, F. H., J. Nutr. *69*, 338–342 (1959).
141. Young, R. J., and R. L. Garrett, J. Nutr. *81*, 321–329 (1963).
142. Fields (Gellei), M., and S. Gatt, Nature *198*, 994–995 (1963).
143. Gatt, S., and B. Shapiro, Nature *185*, 461–462 (1960).
144. Gatt, S., Biochim. Biophys. Acta *70*, 370–380 (1963).
145. Stein, Y., and B. Shapiro, Am. J. Physiol. *196*, 1238–1241 (1959).
146. Goodman, D. S., J. Am. Chem. Soc. *80*, 3892–3898 (1958).
147. Craig, B. M., Can. J. Technol. *34*, 335–339 (1956).
148. Brown, H. B., and I. H. Page, Lancet *i*, 152–153 (1959).
149. Beare, J. L., Food Manuf. *32*, 378–384 (1957).
150. Thomasson, H. J., J. Nutr. *59*, 343–352 (1956).
151. Hopkins, C. Y., M. J. Chisholm, T. K. Murray and J. A. Campbell, JAOCS *34*, 505–508 (1957).
152. Wagner, H., E. Seelig and K. Bernhard, Hoppe-Seylers Z. Physiol. Chem *312*, 104–110 (1958).
153. Bernhard, K., F. Lindlar and H. Wagner, Z. Ernahrungswiss *1*, 48–54 (1960).
154. Carroll, K. K., Can. J. Biochem. Physiol. *40*, 1115–1122 (1962).
155. Dailey, R. E., L. Swell, H. Field, Jr., and C. R. Treadwell, Proc. Soc. Exp. Biol. Med. *105*, 4–6 (1960).
156. Gidez, L. I., Biochem. Biophys. Res. Commun. *14*, 413–418 (1964).
157. Carroll, K. K., unpublished observations.
158. Carroll, K. K., Can. J. Biochem. Physiol. *40*, 1229–1238 (1962).
159. Carroll, K. K., JAOCS *40*, 413–419 (1963).
160. Richardson, T., A. L. Tappel, L. M. Smith and C. R. Houle, J. Lipid Res. *3*, 344–350 (1962).
161. Reiser, R., B. Stevenson, M. Kiyama, R. B. R. Choudbury and D. W. Wood, JAOCS *40*, 507–513 (1963).
162. Farkas, T., and S. Herodek, J. Lipid Res. *5*, 369–373 (1964).
163. Mabrouk, A. F., and J. B. Brown, JAOCS *33*, 98–102 (1956).
164. Hartman, L., and F. B. Shorland, Nature *184*, 2024 (1959).
165. Johnston, P. V., O. C. Johnston and F. A. Kummerow, Science *126*, 698–699 (1957).
166. Johnston, P. V., O. C. Johnston and F. A. Kummerow J. Nutr. *65*, 13–23 (1958).
167. Johnston, P. V., O. C. Johnston and F. A. Kummerow, Proc. Soc. Exp. Biol. Med. *96*, 760–762 (1957).
168. Kaufmann, H. P., and G. Mankel, Fette, Seifen, Anstrichmittel *66*, 6–13 (1964).
169. Sinclair, R. G., J. Biol. Chem. *111*, 515–526 (1935).
170. McConnell, K. P., and R. G. Sinclair, J. Biol. Chem. *118*, 131–136 (1937).
171. Kohl, M. F. F., J. Biol. Chem. *126*, 709–719 (1938).
172. Raulin, J., C. Loriette and G. Clement, Biochim. Biophys. Acta *70*, 642–646 (1963).
173. Dhopeshwarkar, G. A., and J. F. Mead, J. Lipid Res. *3*, 238–242 (1962).
174. Blank, M. L., and O. S. Privett, J. Lipid Res. *4*, 470–476 (1963).
175. Knipprath, W. G., and J. F. Mead, JAOCS *41*, 437–440 (1964).
176. Coots, R. H., J. Lipid Res. *5*, 473–476 (1964).
177. Holman, R. T., Proc. Soc. Exp. Biol. Med. *76*, 100–102 (1951).
178. Privett, O. S., F. J. Pusch and R. T. Holman, Arch. Biochem. Biophys. *57*, 156–162 (1955).
179. Holman, R. T., and E. Aaes-Jorgensen, Proc. Soc. Exp. Biol. Med. *93*, 175–179 (1956).
180. Mattson, F. H., J. Nutr. *71*, 366–370 (1960).
181. Christensen, F., H. Dam and F. P. Engel, Acta Physiol. Scand. *38*, 373–378 (1957).
182. Aaes-Jorgensen, E., J. Nutr. *66*, 465–483 (1958).
183. Miller, E. S., and G. O. Burr, Proc. Soc. Exp. Biol. Med. *36*, 726–729 (1937).
184. Cruickshank, E. M., J. Houston and T. Moore, Biochem. J. *33*, 1630–1634 (1939).
185. Reiser, R., Arch. Biochem. Biophys. *32*, 113–120 (1951).
186. Moore, T., and I. M. Sharman, Biochem. J. *81*, 10P (1961).
187. Meade, E. M., in "Progress in the Chemistry of Fats and Other Lipids," Vol. 4, ed. R. T. Holman, W. O. Lundberg and T. Malkin, Pergamon Press, New York, 1957, pp. 47–62.
188. Mikolajczak, K. L., F. R. Earle and I. A. Wolff, JAOCS *40*, 342–343 (1963).
189. Bernhard, K., and U. Gloor, Helv. Chim. Acta *36*, 296–299 (1953).
190. Dear, R. E. A., and F. L. M. Pattison, J. Am. Chem. Soc. *85*, 622–626 (1963).
191. Assilineau, J., and E. Lederer, in "Lipide Metabolism," ed. K. Bloch, J. Wiley and Sons, Inc., New York, 1960, pp. 337–406.
192. Assilineau, J., "Les lipides bacteriens," Hermann, Paris, 1962.
193. O'Leary, W. M., Bacteriol. Rev. *26*, 421–447 (1962).
194. Abrahamsson, S., S. Stallberg-Stenhogen and E. Stenhogen, in "Progress in the Chemistry of Fats and Other Lipids," Vol. 7, Part I, ed. R. T. Holman and T. Malkin, Pergamon Press, New York, 1963. pp. 1–164.
195. Downing, D. T., J. Lipid Res. *5*, 210–215 (1964).
196. Livingston, M., M. E. Bell, F. B. Shorland, T. Gerson and R. P. Hansen, Biochem. J. *65*, 438–440 (1957).
197. Gerson, T., F. B. Shorland, Y. Adams and M. E. Bell, Biochem. J. *73*, 594–596 (1959).
198. Bergström, S., B. Borgström, N. Tryding and G. Westöö, Biochem. J. *58*, 604–608 (1954).
199. Tryding, N., and G. Westöö, Acta Chem. Scand. *10*, 1234–1242 (1956).
200. Tryding, N., and G. Westöö, Acta Chem. Scand. *11*, 427–438 (1957).
201. Tryding, N., and G. Westöö, Arkiv. Kemi. *11*, 291–305 (1957).
202. Stern, H. S., and C. R. Treadwell, Proc. Soc. Exp. Biol. Med. *97*, 579–581 (1958).
203. Goodman, D. S., and D. Steinberg, J. Biol. Chem. *233*, 1066–1071 (1958).
204. Preiss, B., and K. Bloch, J. Biol. Chem. *239*, 85–88 (1964).
205. Hofmann, K., "Fatty Acid Metabolism in Microorganisms," J. Wiley and Sons, Inc., New York, 1963.
206. Evans, R. J., S. L. Bandemer, M. Anderson and J. A. Davidson, J. Nutr. *76*, 314–319 (1962).
207. Ellis, N. R., C. S. Rothwell and W. O. Pool, Jr., J. Biol. Chem. *92*, 385–398 (1931).
208. Reiser, R., B. Stevenson, M. Kayama, R. B. R. Choudbury and D. W. Hood, JAOCS *40*, 507–513 (1962).
209. Reiser, R., and P. K. Raju, Biochem. Biophys. Res. Commun. *17*, 8–11 (1964).
210. Kircher, H. W., JAOCS *41*, 4–8 (1964).
211. Chalvardjian, A., L. J. Morris and R. T. Holman, J. Nutr. *76*, 52–58 (1962).
212. Krewson, C. F., and F. E. Luddy, JAOCS *41*, 134–136 (1964).
213. Rahm, J. J., and R. T. Holman, J. Lipid Res. *5*, 169–176 (1964).
214. Holman, R. T., in "Progress in the Chemistry of Fats and Other Lipids," Vol. 2, ed. R. T. Holman, W. O. Lundberg and T. Malkin, Academic Press, New York, 1954, pp. 51–98.
215. Privett, O. S., JAOCS *36*, 507–512 (1959).
216. Privett, O. S., and M. L. Blank, JAOCS *39*, 465–469 (1962).
217. Tappel, A. L., W. D. Brown, H. Zalkin and V. P. Maier, JAOCS *38*, 5–9 (1961).
218. Alfin-Slater, R. B., and R. S. Morris, in "Advances in Lipid Research," Vol. 1, ed. R. Paoletti and D. Kritchevsky, Academic Press, New York, 1963, pp. 183–210.
219. Mead, J. F., in "Adipose Tissue as an Organ," ed. L. W. Kinsell, Charles C. Thomas, Springfield, Ill., 1962, pp. 148–149.
220. Bhaleroo, V. R., M. Inoue and F. A. Kummerow, J. Dairy Sci. *46*, 176–180 (1963).
221. Kaunitz, H., C. A. Slanetz, R. E. Johnson and S. F. Herb, JAOCS *38*, 301–305 (1961).
222. Perkins, E. G., J. G. Endres and F. A. Kummerow, Proc. Soc. Exp. Biol. Med. *106*, 370–372 (1961).
223. Swern, D., in "Progress in the Chemistry of Fats and Other Lipids," Vol. 3, ed. R. T. Holman, W. O. Lundberg and T. Malkin, Pergamon Press, New York, 1955, pp 213–241.
224. Mikolajczak, K. L., F. R. Earle and I. A. Wolff, JAOCS *39*, 78–80 (1962).
225. Diamond, M. J., R. E. Knowles, R. G. Binder and L. A. Goldblatt, JAOCS *41*, 430–433 (1964).
226. Binder, R. G., T. H. Applewhite, G. O. Kohler and L. A. Goldblatt, JAOCS *39*, 513–517 (1962).
227. Perkins, E. G., J. G. Endres and F. A. Kummerow, J. Nutr. *73*, 291–298 (1961).
228. Uchiyama, M., R. Sato and M. Mizugaki, Biochim. Biophys. Acta *70*, 344–346 (1963).
229. Watson, W. C., and R. S. Gordon, Jr., Biochem. Pharmacol. *11*, 229–236 (1962).
230. Kaunitz, H., and R. E. Johnson, JAOCS *41*, 50–52 (1964).
231. Holman, R. T., W. O. Caster and H. F. Wiese, Amer. J. Clin. Nutr. *14*, 70–75 (1964).
232. Holman, R. T., W. O. Caster and H. F. Wiese, Amer. J. Clin. Nutr. *14*, 193–196 (1964).
233. Hansen, A. E., H. F. Wiese, A. N. Boelsche, M. F. Haggard, D. J. D. Adams and H. Davis, Pediatrics *31*, 171–192 (196).
234. Noble, R. L., K. K. Carroll and A. S. Douglas, Can. J. Med. Assoc. J. *76*, 23–26 (1957).
235. Silversides, J. L., Can. Med. Assoc. J. *86*, 537–539 (1962).
236. Fleischer, S., this Symposium.
237. Vandenheuvel, F. A., this Symposium.
238. Vandenheuvel, F. A., JAOCS *40*, 455–471 (1963).
239. Richardson, R., A. L. Tappel, L. M. Smith and C. R. Houle, J. Lipid Res. *3*, 344–350 (1962).
240. Robertson, A. F., and W. E. M. Lands, J. Lipid Res. *5*, 88–93 (1964).
241. Walker, B. L., and F. A. Kummerow, J. Nutr. *82*, 323–328 (1964).
242. Walker, B. L., and F. A. Kummerow, Proc. Soc. Exp. Biol. Med. *115*, 1099–1103 (1964).
243. Symposium on Fats in Human Nutrition, J. Am. Med. Assoc. *164*, 1890–1925 (1957).
244. "Chemistry of Lipids as related to Atherosclerosis." ed. I. H. Page, Charles C. Thomas, Publishers, Springfield, Ill., 1958.
245. Symposium on Lipids in Health and Disease, Fed. Proc. *20*, Suppl. 7, 115–162 (1961).
246. Kinsell, L. W., in "Progress in Chemistry of Fats and Other Lipids," Vol. 6, ed. R. T. Holman, W. O. Lundberg and T. Malkin, Pergamon Press, New York, 1963, pp. 137–170.
247. Klein, P. D., and R. A. Martin, J. Biol. Chem. *234*, 1685–1687 (1959).
248. Goodman, D. S., and T. Shiratori, Biochim. Biophys. Acta *84*, 104–106 (1964).
249. Goodman, D. S., D. Deykin and T. Shiratori, J. Biol. Chem. *239*, 1335–1345 (1964).
250. Wright, A. S., G. A. J. Pitt and R. A. Morton, Lancet *2*, 594–597 (1959).
251. Boettcher, C. J. F., and C. M. van Gent, J. Atherosclerosis Res. *1*, 34–46 (1961).
252. Boettcher, C. J. F., and F. P. Woodford, J. Atherosclerosis Res. *1*, 434–443 (1961).
253. Deuel, H. J., in "Progress in the Chemistry of Fats and Other Lipids," Vol. 2, ed. R. T. Holman, W. O. Lundberg and T. Malkin, Academic Press, Inc., New York, 1954, pp. 99-192.
254. Murray, T. K., J. L. Beare, J. A. Campbell and C. Y. Hopkins, Can. J. Biochem. Physiol. *36*, 653–657 (1958).