The Metabolism of the Essential Fatty Acids

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PRIOR to the advent of isotopic tracers, evidence on the metabolism of the polyunsaturated fatty acids was obtained by alkali-isomerization analysis of tissue fats following the feeding of various fatty acids. This method, while it gave only indirect evidence, still furnished some interesting information. For instance, the feeding of a dienoic acid (linoleic) resulted in increases in dienoic and tetraenoic acids in the animal's tissues.[1,2,3] Feeding of trienoic acid (linolenic) resulted in increases in trienoic, pentaenoic and hexaenoic acids.[1,2,3] When animals were placed on a fat-free diet, increases in a trienoic acid were noted.[1,4,5] Other types of experiments gave additional information on the metabolic transformations of the essential fatty acids. For example, Bernhard and Schoenheimer[6] found that linoleic acid does not incorporate deuterium from the body water of animals although the saturated fatty acids and oleic acid incorporate fairly large amounts. Thus, the available evidence has indicated, first, that the essential fatty acids are not readily synthesized in the animal body (which could already be surmised from their nature) and second, that when obtained from an outside source, they can undergo various transformations.

The availability of fatty acids labeled with C^{14} in the carboxy group[7,8] and of methods for the separation and degradation of these fatty acids[9,10] has enabled many of the earlier results to be explained and the metabolism of the polyunsaturated fatty acids to be elucidated.

Acids were fed as methyl esters to rats on a fat-free diet. After four hours, the rats were killed, and their organ and abdominal depot fat was hydrolyzed to obtain the fatty acids. These were crystallized to separate saturated and unsaturated fractions, and the polyunsaturated fatty acids were isolated, as the insoluble polybromides, or by chromatography on the reversed phase chromatogram of Howard and Martin.[9] Fatty acids separated in this manner were degraded stepwise,[10] and the location of the C^{14} in the carbon chain was ascertained, where possible.

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LINOLEIC ACID

In the first experiment,[11] carboxy-labeled acetate was injected intraperitoneally. Linoleic acid isolated from these rats as the tetrabromide had a small amount of activity which, however, was almost completely removed by eight recrystallizations. The arachidonic acid from this experiment had essentially all its activity in the carboxy carbon. These results confirmed previous ideas that linoleic acid is not formed to an appreciable extent in the animal body and indicated that arachidonic acid is synthesized by addition of acetate to an 18-carbon exogenous precursor, presumably linoleic acid.

When carboxy-labeled linoleic acid was fed, active linoleic, arachidonic and probably docosapentaenoic acids were isolated.[12] Degradation of the arachidonic acid gave the following results, expressed as per cent of the total activity in each carbon atom:

$$\underset{0}{C_{17}H_{35}}\text{—}\underset{24.5}{CH_2}\text{—}\underset{0.7}{CH_2}\text{—}\underset{74.7}{COOH}$$

The complete absence of activity beyond the third carbon atom confirmed the hypothesis that the linoleic acid molecule is incorporated unchanged into arachidonic acid. The activity in the third carbon atom is undoubtedly that of the carboxy carbon of the linoleic acid. The surprisingly high activity in the carboxy car-

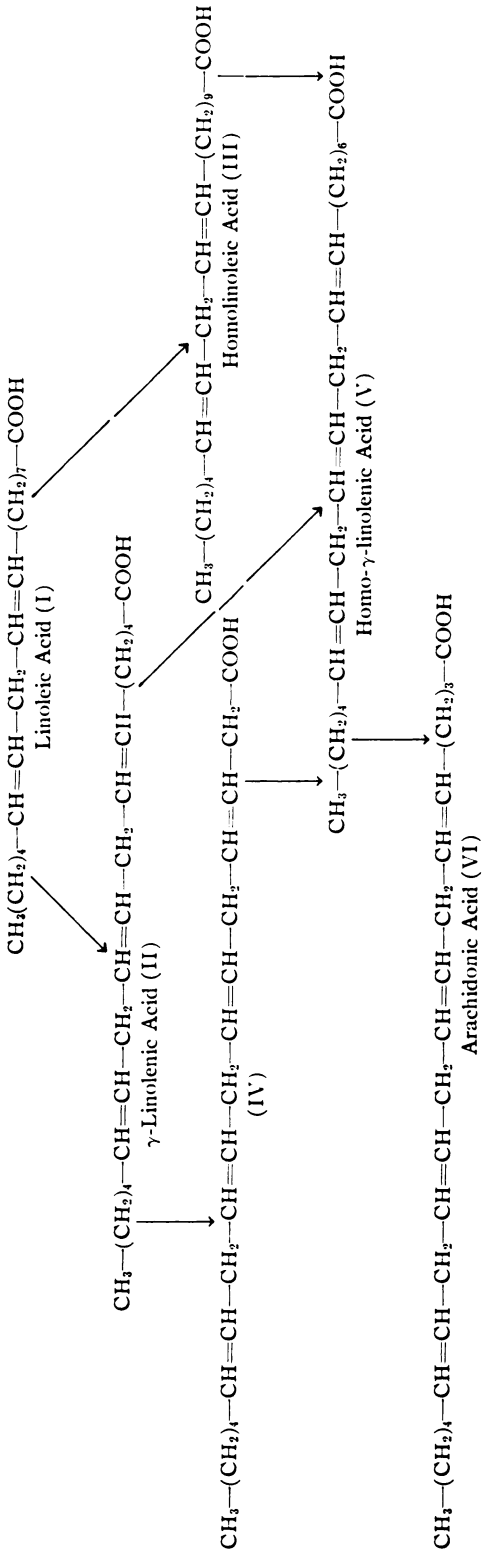

Fig. 1. Possible intermediate compounds in the transformation of arachidonic acid from linoleic acid.

bon undoubtedly comes from acetate formed by degradation of the active linoleic acid. In previous experiments[13] it was shown that fed linoleic acid is very rapidly oxidized. If the active acetic acid thus formed did not immediately mix with the general acetate pool but contributed to the chain-lengthening process without much dilution, a reasonable explanation for the relatively high activity of the carboxy carbon of arachidonic acid is on hand.

While these experiments demonstrated that arachidonic acid is formed in the animal body by addition of acetate to the carboxy end of linoleic acid, they did not indicate the order of events in this 3-step process involving one chain lengthening and two dehydrogenations. The possible intermediate compounds in this transformation are given in Figure 1. However, some evidence was available which aided in determining the most probable first step of the process. Thus, homolinoleic acid was reported by Karrer and Koenig[14] to be devoid of essential fatty acid activity and by Thomasson[15] to possess about 40 per cent of the activity of linoleic acid. Moreover, Thomasson reported that γ-linolenic acid was fully as active as linoleic acid. Hence, inasmuch as an intermediate in such a conversion should possess at least the activity of its immediate precursor, the pathway from I to II was definitely favored over that from I to III.

For these reasons, carboxy-labeled γ-linolenic (6,9,12-octadecatrienoic) acid was prepared from the seeds of *Oenothera Lamarckiana* and fed to rats in the usual manner.[16] In this case, as can be seen in Table I, almost all the activity in the fatty acids was found in the arachidonic acid. Several other interesting points are to be seen in the table. First, the linoleic acid from fraction F-3 (acetone-soluble at −60°) was active. However, linoleic acid isolated as the tetrabromide from fraction F-2 (acetone-insoluble at −60°, soluble at −20°) contained little activity. The activity in that from F-3, therefore, must have been due to a contaminant of similar chromatographic behavior, presumably the fed γ-linolenic acid. If this is true, only trace amounts of the fed acid were present four hours after feeding (its activity was 1.4×10^5 disintegrations per second

TABLE I

Weights and Activities of Lipid Fractions from 1-C^{14}-γ-Linolenate-fed Rats

	Weight g	Specific activity d.p.s.* per mg
Total fat	16.5	(9.5)
Total fatty acids	16.25	41
Fatty acid fraction I (acetone-insoluble at −20°)	4.95	15.2
Fatty acid fraction II (acetone-insoluble at −60°)	8.55	5.6
Fatty acid fraction III (acetone-soluble at −60°)	2.25	235

* d.p.s.—disintegrations per second.

per mg), again indicating almost complete conversion of the fed acid to arachidonic.

A second source of interest (Table II) is in the relative activities of the fed acid, the linoleic acid, the monounsaturated and the saturated acids. From these data, it does not appear that hydrogenation of polyunsaturated acids through the monounsaturated to the unsaturated acids is an important pathway if, indeed, it occurs at all. Finally, it can be seen that the second most active of the biosynthesized acids is a docosapentaenoic or docosahexaenoic acid presumably formed by addition of acetate to arachidonic acid. The distribution of activity

TABLE II

Activities of Fatty Acids from γ-Linolenate-fed Rats

Fraction	Component	Specific activity d.p.s.* per mg
F-1	Palmitic acid	1.9
	Stearic acid	2.5
F-2	Oleic acid	0.37
	Linoleic acid	0.63
F-3	Palmitoleic acid	0.45
	Linoleic acid (contaminated by γ-linolenic)	50.7
	Arachidonic acid	785
	Docosapentaenoic or docosahexaenoic acid	14.9

* d.p.s.—disintegrations per second.

in the arachidonic acid expressed as per cent of total activity is as follows:

$$C_{17}H_{35}—CH_2—CH_2—COOH$$
$$1.2 \quad 97.4 \quad 0.3 \quad 1.1$$

Once again it can be seen that the γ-linolenic acid molecule must have been used almost exclusively for formation of arachidonic acid. The small amount oxidized to acetic acid is shown by the low activity of the carboxy group as well as by the low activity of the saturated fatty acids from the same animals. It might be said, in this respect, that γ-linolenic acid is about the equivalent of arachidonic acid as an essential fatty acid. The activity beyond the third carbon atom deserves notice and will be discussed below.

Thus it appears at the present time that the pathway from linoleic to arachidonic acid is as shown in Figure 2. The last step in the process is under consideration in our laboratory at the present time.

LINOLENIC ACID

It is evident that linolenic acid has no place in the formation of arachidonic from linoleic acid. Experiments in which carboxy-labeled linolenic acid was fed to rats gave some information on the metabolism of this acid.[17] Both the C_{20} acid and the C_{22} acid (docosapentaenoate or docosahexaenoate) isolated from these animals were quite active. Linoleic acid, purified by chromatography of the tetrabromide, was only about 0.3 per cent as active as the arachidonic acid, indicating clearly that a pathway from linolenic to linoleic acid is very minor at most. The distribution of activity in the arachidic acid derived by hydrogenation of the C_{20} fraction was as follows:

$$C_{16}H_{33}—CH_2—CH_2—CH_2—COOH$$
$$1.0 \quad 3.8 \quad 60.2 \quad 7.8 \quad 27.2$$

First it can be seen that linolenic acid is intermediate between linoleic and γ-linolenic acids in the relative rates of oxidation and incorporation into higher polyenoic acids. Second, some activity is again seen beyond the third carbon. It seems likely that this activity is due to partial degradation and resynthesis of the acids at all stages. The activity in the even carbons cannot be explained at present.

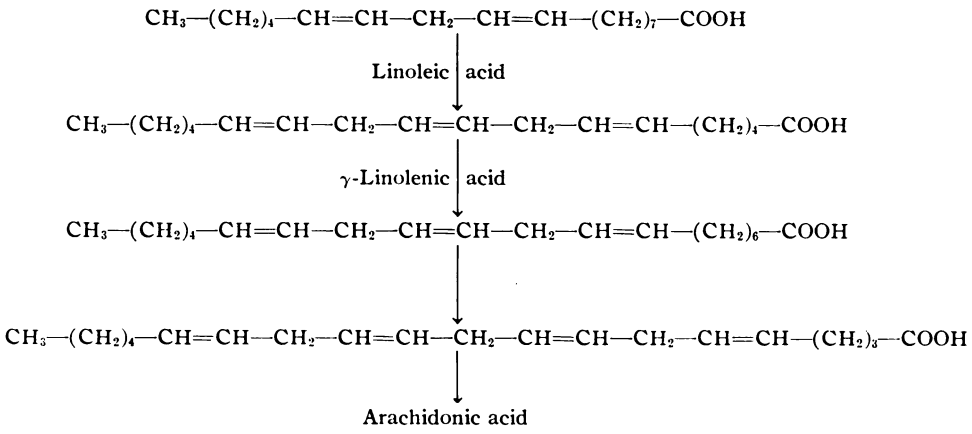

Fig. 2. Pathway of linoleic to arachidonic acid.

Since linolenic acid does not behave as a typical essential fatty acid in feeding experiments, a question arises as to the identity of the active C_{20} acid formed from it. Several observations would indicate that it is not arachidonic acid. First, although chromatography did not completely separate the unsaturated acids, a partial separation was made in which the leading portion (containing docosapentaenoic or docosahexaenoic acids) was arbitrarily separated from the trailing portion (containing the arachidonic acid). When this was done, it was found that the radioactivity was concentrated in the leading portion. Second, alkali isomerization experiments showed that more pentaene and hexaene were formed in the rats which had been fed linolenate. Finally in a separate experiment, fat-deficient rats were fed methyl linolenate and their organ and depot fatty acids were analyzed chromatographically.[18] The fatty acids of rats on the fat-free diet contained no 22-carbon acids whereas those from the linolenate-fed rats contained an appreciable amount of these acids but gave no evidence for linolenic acid. Evidently, the linolenate was rapidly converted to a C_{22} acid. A proposed metabolic pathway for linolenic acid is illustrated in Figure 3.

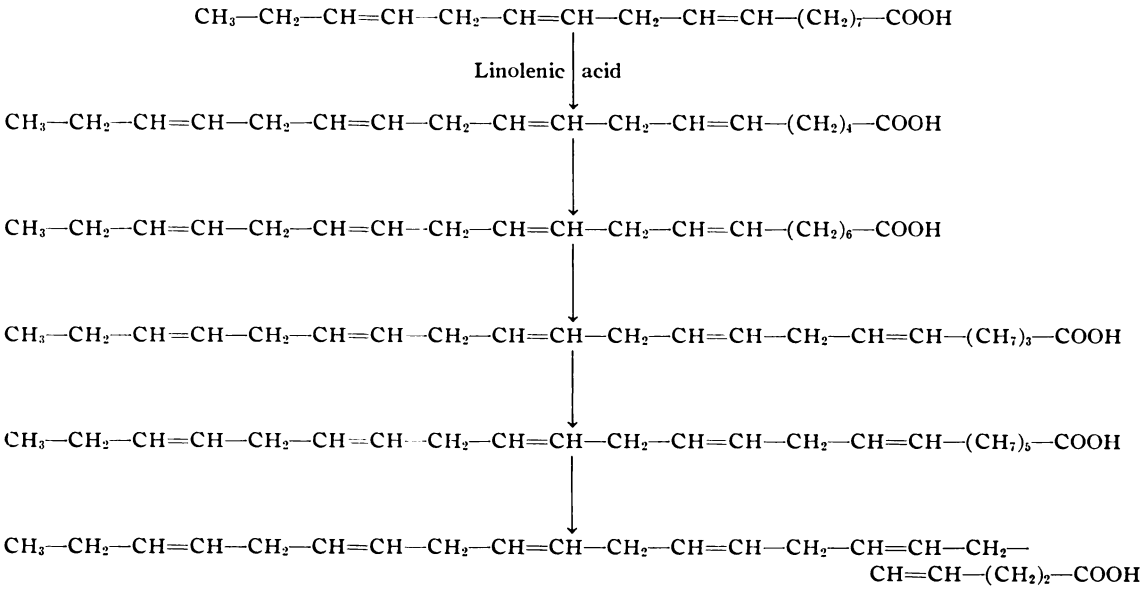

Fig. 3. Proposed metabolic pathway of linoleic acid.

Although these observations serve to explain the earlier data on the changes induced in body fat by ingestion of the dienoic and trienoic acids, they do not explain the appearance of the trienoic acid typical of the onset of the fat-deficiency state. Isolation and characterization of this acid from fat-deficient rats revealed it to be 5,8,11-eicosatrienoic acid.[19] Although it appeared at first that it might be a hydrogenation product of arachidonic acid (the double bond farthest from the carboxy end is lacking), a recent suggestion has been made that it is actually a dehydrogenation product of oleic acid.[20] If this idea proves correct, it seems likely that in the absence of the normal substrates, linoleic or linolenic acids, the unsaturated fatty acid dehydrogenase enzymes use the readily available oleic acid as a substrate. That the product is not an efficient essential fatty acid is attested by the fact that the triene increases concurrently with the severity of the fat-deficiency symptom. In any event, a metabolic pathway may now be proposed similar to those probably followed by linoleic and linolenic acids (Figure 4).

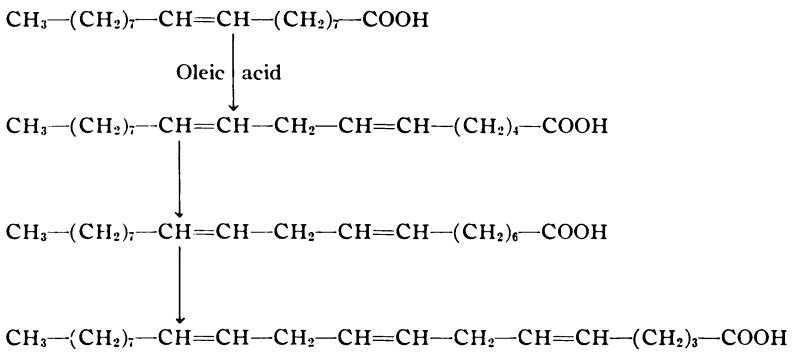

Fig. 4. Proposed pathway of oleic acid to a trienoic acid.

CONCLUSION

If these hypotheses are shown to be correct, the polyunsaturated fatty acids of the animal body can be said to be formed by successive additions of double bonds in the divinyl methane relationship to the existing double bonds of unsaturated fatty acids ingested or synthesized from saturated acids in the body. The new double bonds are introduced between the existing double bonds and the carboxy groups of the acids which may be lengthened by addition of acetate when the double bond system approaches to within three or four methylenes of the carboxy group. There thus arise three "families" of polyunsaturated fatty acids, not readily interconvertible and possibly serving different functions in the body.

REFERENCES

1. Rieckehoff, I. G., Holman, R. T., and Burr, G. O.: Polyethenoid fatty acid metabolism. Effect of dietary fat on polyethenoid fatty acids of rat tissues. *Arch. Biochem.* 20: 331, 1949.
2. Widmer, C., Jr. and Holman, R. T.: Polyethenoid fatty acid metabolism. II. Disposition of polyunsaturated fatty acids in fat-deficient rats upon single fatty acid supplementation. *Arch. Biochem.* 25: 1, 1950.
3. Reiser, R.: The metabolism of polyunsaturated fatty acids in growing chicks. *J. Nutrition* 42: 325, 1950.
4. Reiser, R.: The synthesis and interconversions of polyunsaturated fatty acid by the laying hen. *J. Nutrition* 44: 159, 1951.
5. Klein, P. D. and Johnson, R. M.: A study of the onset of unsaturated fatty acid deficiency in subcellular particles of rat livers. *Arch. Biochem.* 48: 380, 1954.
6. Bernhard, K. and Schoenheimer, R.: The inertia of highly unsaturated fatty acids in the animal, investigated with deuterium. *J. Biol. Chem.* 133: 707, 1940.
7. Howton, D. R., Davis, R. H., and Nevenzel, J. C.: Unsaturated fatty acids. III. Preparation of 1-C^{14}-linoleic acid. *J. Am. Chem. Soc.* 76: 4970, 1954.
8. Nevenzel, J. C. and Howton, D. R.: Unsaturated fatty acids. IV. Preparation of oleic-1-C^{14} acid. *J. Org. Chem.* 22: 319, 1951.
9. Silk, M. H. and Hahn, H. H.: The resolution of mixtures of C_{16}-C_{24} normal-chain fatty acids by

reversed-phase partition chromatography. *Biochem. J.* 56: 406, 1954.

10. DAUBEN, W. G., HOERGER, E., and PETERSEN, J. W.: Distribution of acetic acid carbon in high fatty acids synthesized from acetic acid by the intact mouse. *J. Am. Chem. Soc.* 75: 2347, 1953.
11. MEAD, J. F., STEINBERG, G., and HOWTON, D. R.: Metabolism of essential fatty acids. Incorporation of acetate into arachidonic acid. *J. Biol. Chem.* 205: 683, 1953.
12. STEINBERG, G., SLATON, W. H., JR., HOWTON, D. R., and MEAD, J. F.: Metabolism of essential fatty acids. IV. Incorporation of linolenate into arachidonic acid. *J. Biol. Chem.* 220: 257, 1956.
13. MEAD, J. F., SLATON, W. H., JR., and DECKER, A. B.: Metabolism of essential fatty acids. II. Metabolism of stearate, oleate and linoleate by fat-deficient and normal rats. *J. Biol. Chem.* 218: 401, 1956.
14. KARRER, P. and KOENIG, H.: Beitrag Zur Kenntris der essentiellen ungesattigten Fettsauren. *Helvet. Chim. Acta* 26: 619, 1943.
15. THOMASSON, H. J.: Biological standardization of essential fatty acids (a new method). *Rev. Vitamin Res.* 25: 1, 1953.
16. MEAD, J. F. and HOWTON, D. R.: Metabolism of essential fatty acids. VII. Conversion of γ-linolenic acid to arachidonic acid. *J. Biol. Chem.* 219: 705, 1956.
17. STEINBERG, G., SLATON, W. H., JR., HOWTON, D. R., and MEAD, J. F.: Metabolism of essential fatty acids. V. Metabolic pathway of linolenic acid. *J. Biol. Chem.* 224: 841, 1957.
18. MEAD, J. F.: The metabolism of the essential fatty acids. VI. Distribution of unsaturated fatty acids in rats on fat-free and supplemented diets. *J. Biol. Chem.* 227: 1025, 1957.
19. MEAD, J. F. and SLATON, W. H., JR.: Metabolism of essential fatty acids. III. Isolation of 5,8,11-eicosatrienoic acid from fat-deficient rats. *J. Biol. Chem.* 219: 705, 1956.
20. MONTAG, W., KLENK, E., HAYES, H., and HOLMAN, R. T.: Eicosapolyenoic acids occurring in the glycerophosphatides of beef liver. *J. Biol. Chem.* 227: 53, 1957.