The Metabolism of the Polyunsaturated Fatty Acids

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FROM time to time during the past few years current knowledge of the metabolism of the polyunsaturated acids has been reviewed. Each time there have been included bits of information gathered since the last review which bring us somewhat nearer our goal: the complete elucidation of the formation and fate of these acids. Although it cannot truthfully be said that we have achieved this goal, still, with a little speculation, we can at last begin to see the whole picture rather clearly.

Most of the early evidence on the transformations of the polyunsaturated acids was obtained with the technic of alkaline isomerization. This technic gave an indication of the alterations in the unsaturated centers of the fatty acids of various tissues but gave little or no information on the alterations of the fatty acids themselves or their transformation to one another. However, interesting and provocative evidence was obtained.

For example, it was found that when a dienoic acid (linoleic) was fed to rats on a fat-free diet, the body fatty acids showed an increase in diene and tetraene;[1,2] when a trienoic acid (linolenic) was fed, there was an increase in triene, pentaene and hexaene acids in the body fat;[2,3] when the fat-free diet was continued for some time, there was an increase in triene.[1,4] In starting our studies of the metabolism of essential fatty acids we addressed ourselves principally to the interpretation of these observations.

The first experiment, which was chosen both for simplicity and because it was basic to the succeeding experiments, involved injection of carboxy-labeled acetate into young rats.[5] The organ and depot fat of these rats yielded fatty acids which were separated by crystallization at low temperature into saturated and unsaturated fractions. From the latter were obtained, by precipitation of the polybromides and chromatographic separation on a rubber column, the linoleic and (somewhat impure) arachidonic acids. The linoleic acid was found to contain essentially no activity. One-step degradation of the arachidonic acid revealed that essentially the activity resided in the carboxy group.

From these results, two conclusions were drawn. First, linoleic acid is not synthesized from acetate by the rat; second, arachidonic acid is synthesized from acetate and an exogenous C_{18} precursor.

The second experiment was designed to confirm our supposition that this exogenous precursor was indeed linoleic acid.[6] Carboxy-labeled methyl linoleate was fed to mature rats, and, after four hours, the rats were killed and the polyunsaturated acids were again isolated as the bromides and separated chromatographically, this time on the reversed-phase column of Howard and Martin. The arachidonic acid obtained from this column had been hydrogenated to arachidic acid during the separation procedure. This acid was degraded stepwise to obtain the first three carbon atoms (as benzoic acid) and the remaining seventeen

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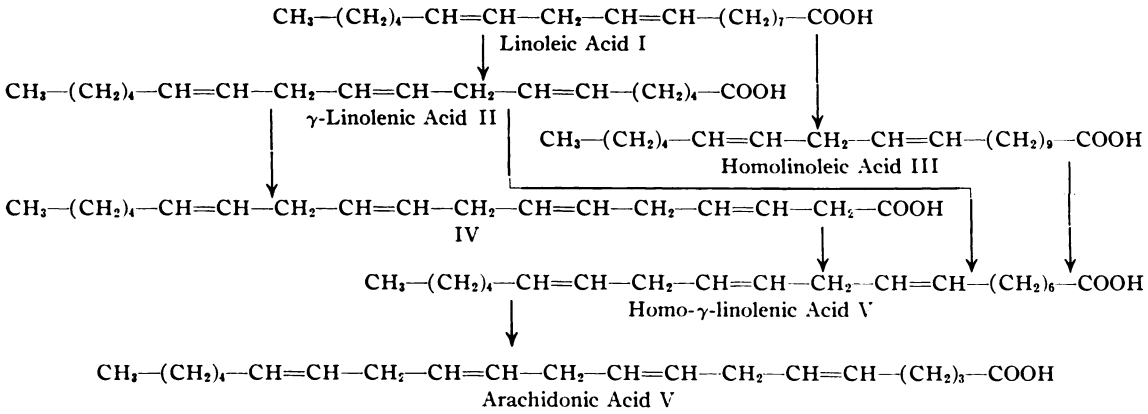


FIG. 1. Possible steps in synthesis of arachidonic acid.

carbons. The distribution of activity in the original arachidonic acid follows:

	$C_{17}H_{35}$—	CH_2—	CH_2—	COOH
% of total activity	0	24.5	0.7	74.7

Several conclusions were drawn from this experiment. First, since the terminal eighteen carbons have the same distribution of activity as the fed (carboxy-labeled) linoleic acid, the postulated eighteen-carbon exogenous precursor of arachidonic acid is indeed linoleic acid. Second, as was predicted from the results of the first experiment, acetate derived from catabolism of the linoleic acid contributed activity to the carboxy group of arachidonic acid. Third, the relative activity of the carboxy group of arachidonic acid is greater than was expected. This last observation will be re-emphasized later.

TABLE I

Activities of Fatty Acids from Rats Fed γ-Linolenate

Fraction	Acid Component	Specific Activity (disintegrations per second per mg.)
F-1	Palmitic	1.9
	Stearic	2.5
F-2	Oleic	0.37
	Linoleic	0.63
F-3	Palmitoleic	0.45
	Linoleic (contaminated by γ-linolenic)	50.7
	Arachidonic	785
	Docosapentaenoic and docosahexaenoic	14.9

The knowledge that arachidonic acid is formed by addition of acetate to linoleic acid did not completely elucidate the biosynthetic pathway, since three steps are involved (two dehydrogenations and a chain-lengthening) and their sequence was not revealed by the experiments previously described. The possible steps in this synthesis are illustrated in Figure 1.

The choice of pathway to follow was made on the basis of the experiment of Karrer and Koenig,[7] who found that homolinoleic acid did not serve as an essential fatty acid in rats and of Thomasson,[8] who found it to possess only 40 per cent of the activity of linoleic acid in prevention of essential fatty acid deficiency. Since an intermediate in a biosynthetic scheme should possess at least the biological activity of its immediate precursor, the pathway through γ-linolenic acid was favored and was chosen for further investigation. This acid was obtained from the seeds of Oenothera Lamarckiana, a relative of the evening primrose, and was labeled in the carboxy group and fed to rats.[9] The arachidonic acid derived from their organ and depot fats was separated by crystallization at low temperature and was degraded stepwise as before. Table I gives the activity of various fatty acids from these animals.

Several points are noteworthy. First, by far the most active acid is arachidonic, indicating the direct and immediate transformation

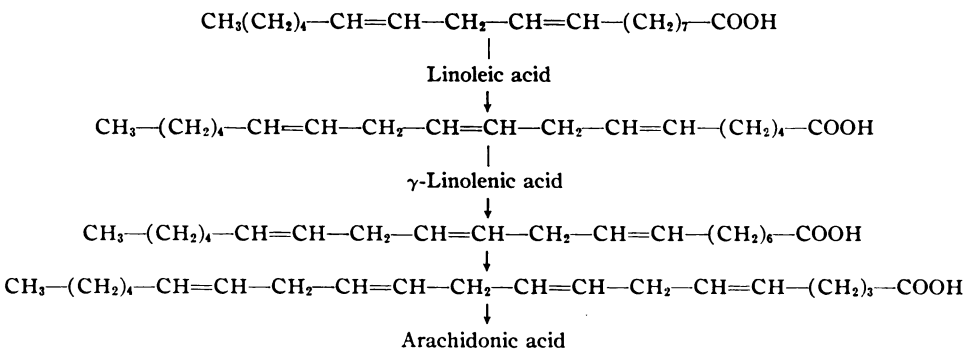


FIG. 2. Proposed scheme for pathway of linoleic to arachidonic acid.

of γ-linolenic acid to arachidonic acid. Second, an impurity in the linoleic acid from fraction F-3 (soluble in acetone at −60°C.) contributed some activity to it as shown by the very low true activity of linoleic acid as derived from F-2 (soluble at −20°C., insoluble at −60°C.). This impurity is evidently largely the fed γ-linoleic acid, which, considering its very high activity, must have been present in vanishingly small amounts. Third, the fact that the saturated acids, palmitic and stearic, are more active than their unsaturated counterparts, palmitoleic, oleic and linoleic acids, argues against hydrogenation as a significant pathway in fatty acid metabolism.

The distribution of activity in the arachidonic acid was as follows:

	$C_{17}H_{35}$—CH_2—CH_2—COOH			
% of total activity	1.2	97.4	0.3	1.1

The very high activity in the third carbon confirms the rapid and complete conversion of γ-linolenic acid to arachidonic acid. The low activity in the first carbon and the low but definite activity beyond carbon 3 will be discussed subsequently.

It thus appears that the preferred pathway from linoleic to arachidonic acid lies through γ-linolenic acid. A proposed scheme for this pathway is shown in Figure 2.

The next-to-last step (conversion of homo γ-linolenic acid to arachidonic) is now under investigation.

In a similar experiment, carboxy-labeled linolenic acid was fed, and the twenty-carbon polyunsaturated fatty acids were isolated from the organ and depot fats of the rats.[10] Degradation of this acid revealed the distribution of activity as follows:

	$C_{16}H_{33}$—CH_2—CH_2—CH_2—COOH				
% of total activity	1.05	3.8	60.2	7.8	27.2

It will be noted, first, that this distribution is intermediate between those of the previous examples and second, that there is considerable activity beyond the third carbon. The activity in the even carbons cannot be explained as yet.

The question arose as to whether or not the twenty-carbon active acid responsible for these data was indeed arachidonic. Several pieces of evidence indicate that it was not. First, a rough chromatographic separation revealed that the most active acid did not follow the distribution of arachidonic acid, but of eicosapentaenoic acid. Second, alkaline isomerization showed an increase in pentaene and hexane. Third, separate experiments, in which linolenate was fed to fat-deficient rats revealed that a docosapentaenoic acid very quickly appeared in their organ and depot fats but not in those of animals fed linoleic acid.[11] It was also noted in these last experiments that four hours after feeding linolenic acid, none could be found in the rats' tissues. Thus it appears that linolenic acid goes directly and rapidly to eicosapentaenoic and docosapentaenoic acids and, possibly, to a docosahexaenoic acid. A

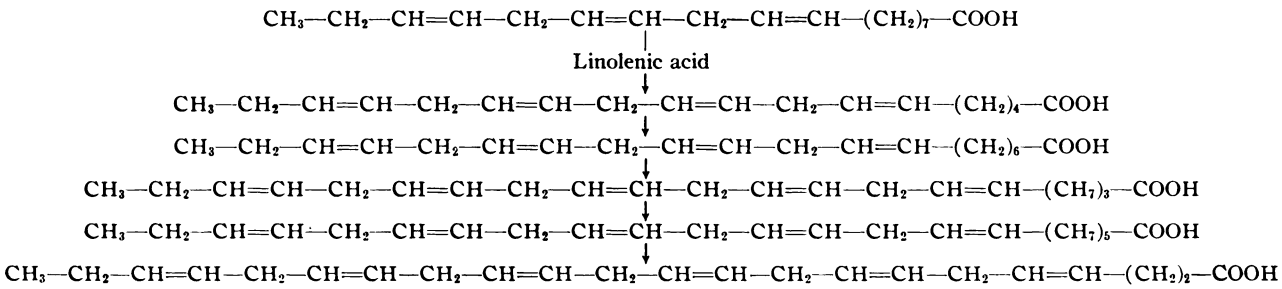


FIG. 3. Transformation of linolenic acid. See text.

possible pathway for such a transformation is shown in Figure 3.

Thus, these experiments have explained the first two alkaline isomerization findings. The third, formation of trienoic acid in animals on a fat-free diet, has also been investigated.

This acid was first isolated as the hexabromide from fat-deficient rats and was ozonized and identified by paper chromatography of the dibasic acid (glutaric) to be revealed as 5:8:11-eicosatrienoic acid[12] as shown in Figure 4.

and, in four hours, their organ and depot fats were removed and converted to fatty acids. The polyunsaturated acids were obtained by crystallization at low temperature and a C_{20} concentrate was derived from these by gas chromatography. From this concentrate was derived, by reversed-phase chromatography, an eicosatrienoic fraction. A portion of this was oxidized with permanganate to a mixture of mono- and dicarboxylic acids and a sample of oleic acid derived from these same animals was

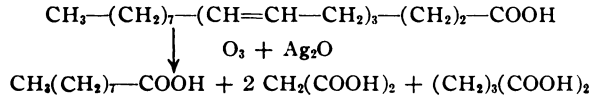


FIG. 4. Degradation of 5:8:11 eicosatrienoic acid.

From this structure, two possible origins suggest themselves: from arachidonic acid by hydrogenation of the terminal double bond or from oleic acid by hydrogenation and chain-lengthening, in a manner similar to the biosynthesis of arachidonic acid. In order to test these alternate hypotheses, fat-deficient rats were injected with carboxy-labeled acetate

TABLE II
Monocarboxylic Acids Resulting from Oxidation of Trienoic Concentrate

Acid	Carbon Atoms	Mole Per Cent	
		Actual	Corrected for Over-oxidation
Decanoic	10	Trace	Trace
Nonanoic	9	82.8	92.7
Octanoic	8	9.0	0
Heptanoic	7	7.5	7.3
Hexanoic	6	0.7	0

treated in the same manner. Second portions of both acids were hydrogenated and degraded stepwise as in previous experiments. That

TABLE III
Dicarboxylic Acids Resulting from Oxidation of Trienoic Concentrate

Acid	Carbon Atoms	Mole Per Cent	
		Actual	Corrected for Over-oxidation
Azelaic	9	5.5	5.5
Suberic	8	4.9	4.9
Pimelic	7	7.1	7.1
Adipic	6	4.0	(4.0)*
Glutaric	5	70.8	81–82.5
Succinic	4	11.7	0–1.5

* Adipic acid was shown by control experiments to be an artifact arising from the polyadipate packing of the gas chromatographic column. Hence it was disregarded in calculating the mole percentage of the other dicarboxylic acids.

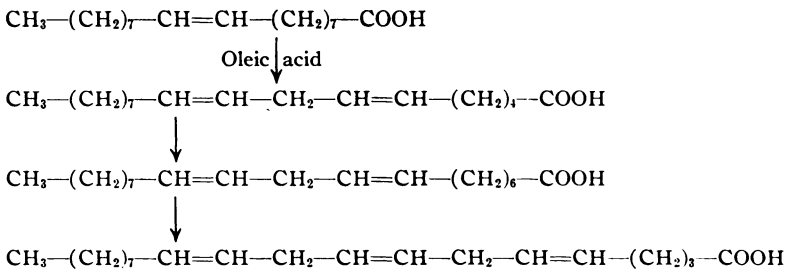
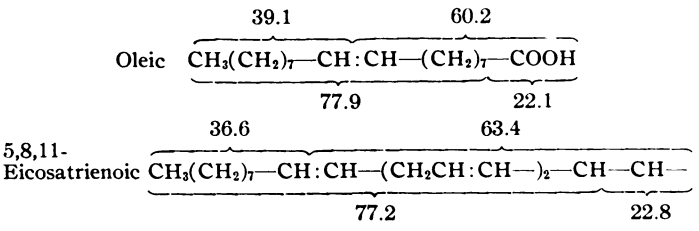


FIG. 5. Transformation of oleic acid. See text.

the oleic acid contained some impurities was indicated by gas-liquid chromatography, which was also used to purify it. From the purified oleic acid were derived, by oxidation, azelaic and pelargonic acids. The trienoic acid fraction was somewhat more complex. Acids derived by oxidation of this fraction are shown in Tables II and III.

From these tables the following conclusions can be drawn: the only monocarboxylic acids formed primarily by the oxidation were pelargonic (C_9, 92.7 per cent) and heptanoic (C_7, 7.3 per cent). Turning to the dicarboxylic acids, we find only 82.5 per cent glutaric acid, which, with pelargonic, was derived from the major 5:8:11-eicosatrienoic acid. Therefore this acid amounted to only 82.5 per cent of the mixture and about 10.2 per cent of the pelargonic acid was derived from some other source. Azelaic acid (C_9, 5.5 per cent), derived from oleic acid, would account for this portion of it. The remaining 4.7 per cent corresponds to the amount of suberic acid (C_8, 4.9 per cent) and indicates a corresponding amount of 8:11-eicosadienoic acid, a possible intermediate in biosynthesis of the eicosatrienoic acid. The amounts of pimelic acid (C_7, 7.1 per cent) corresponds exactly with the amount of heptanoic acid, and would indicate this amount of 7, 10, 13-eicosatrienoic acid. These considerations are shown in Table IV.

The activities of the various degradation products led to the following distribution:



First, it can be seen from the activity of the terminal half of the eicosatrienoic acid that it was derived, not from arachidonic acid but from acetate, since in the former case, it would have contained no activity. Second, from the ratios of activities of the various portions of the eicosatrienoic acid and the corresponding ratios from oleic acid, we can conclude that 5:8:11-eicosatrienoic acid is synthesized from acetate through oleic acid by the fat-deficient rat. This conclusion leads directly to another, namely, that the transformation of oleic acid to 5,8,11-eicosatrienoic acid follows a pathway similar to that of the transformation of linoleic to arachidonic acid. The postulated pathway is shown in Figure 5.

It will be noted, first, that each step in the pathway corresponds to one in the transformation of linoleic acid and, second, that evidence for the occurrence of one of the postulated intermediates, 8,11-eicosadienoic acid, has been presented.

If, in the fat-deficient rat, oleic acid may be

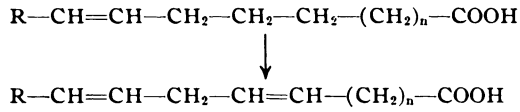
TABLE IV
Composition of Trienoic Concentrate

Acid	Mole Per Cent
5,8,11-Eicosatrienoic	81–82.5
8,11-Eicosadienoic	4.8–5.0
7,10,13-Eicosatrienoic	7.1–7.3
Oleic (9-octadecenoic)	5.4–5.6

transformed into a polyunsaturated acid, a similar transformation may be postulated for palmitoleic acid, which is a major component of the fatty acids of rats in this state.[11] Evidence for such a transformation can be deduced from the probable presence of 7,10,13-eicosatrienoic acid in the mixture. This acid could be derived from palmitoleic acid by steps similar to those postulated above for the other acids.

COMMENTS

From the examples presented it can be seen that the polyunsaturated fatty acids of the animal's body can be formed by successive dehydrogenation and chain-lengthening steps using as starting materials the unsaturated acids obtained through the diet or synthesized from acetate via the saturated acids. The additional double bonds are introduced in the 1:4 relationship to the original double bonds and toward the carboxy group.



If a double bond would logically be introduced into a position $\alpha\ \beta$ or $\beta\ \gamma$ to the carboxy group, apparently two carbons (acetyl coenzyme A) are added to the carboxy end of the molecule and the additional double bond can be introduced in a "safe" position. As substrates for this alteration, use can apparently be made of any unsaturated fatty acid available in which the distance from the existing double bond system to the carboxy group is not too great. At the present time, the following fatty acids have been found to serve: linolenic acid and its products, linoleic acid and its products, oleic and its products and palmitoleic acid and its products. Of these families of acids only the linoleic family has been found to cure deficiency of essential fatty acids. In this case, however, the parent compound may actually be 12-octadecenoic acid, which, by virtue of its low concentration in the dietary or synthetic fat, is generally unavailable as a substrate for further dehydrogenation. Two reports[8,13] have indicated that this acid does not cure the symptoms of deficiency of essential fatty acids. However, a consideration of the reported methods of preparation in both cases leads to the conclusion that it has, in fact, probably not been tested. As products of these alterations can be found, in general, C_{20} acids with three, four and five double bonds and C_{22} acids with five and six double bonds. Some more highly unsaturated acids with greater chain length may be found in the mammalian body, but in very small amounts.

Interpretation of these observations may possibly be made on the basis of interplay of three enzymes.

The first, which could be termed a polydehydrogenase, is concerned with the addition of double bonds toward the carboxy group of unsaturated fatty acids. It may have greatest affinity for C_{18} trienoic acids and for the C_{20} trienoic acid of the linoleic family since these are not found in significant concentrations in animal tissues and, if fed, disappear immediately to give rise to more highly unsaturated products. Next in affinity for this enzyme might be the C_{18} dienes of the linolenic and linoleic families and the C_{20} diene of the oleic family since these acids are found in the tissues and, if fed, can be deposited or used readily for oxidation. A low affinity must exist for the monoenoic acids and affinity for this enzyme must decrease very abruptly with chain lengths above twenty-two carbons.

The second enzyme postulated in these transformations is an acyl transferase which transfers the unsaturated fatty acids (presumably as CoA derivatives) to the methyl group of acetyl CoA, thus accomplishing the chain-lengthening process. Evidence for the existence of such an enzyme has come from many sources: the interconversion of palmitic and stearic acids,[14] the families under present discussion and possibly, the mitochondrial systems observed by several workers[15,16] which would apparently add or subtract acetate but would not synthesize long-chain acids from acetate. It is possible that this enzyme forms part of the normal fatty acid degradation system but that its low affinity for the longer chain acids precludes complete degradation in most cases. Additional evidence for this enzyme in the reverse sense, may be found in the appearance

of C^{14} activity beyond the carboxy group of acids which were fed as the carboxy-labeled molecules.

Finally, it may be postulated that the normal fatty acid degradation system can operate on these unsaturated acids, particularly when they are converted to the $\alpha\beta$ or $\beta\gamma$ unsaturated compounds, which are normal intermediates in the degradation process.

Relative affinities of the various fatty acids for these enzymes could be invoked to explain most of the observations discussed today. For example, the appearance of C_{20} trienes in the animal in the absence of dietary linoleic acid could stem from the fact that with lower concentrations of the preferred substrate, linoleic acid, oleic acid may now compete favorably for the polydehydrogenase and be converted to the acid in question. Likewise, the relative activity of C_1 and C_3 of the C_{20} acids formed from carboxy-labeled linoleic, linolenic and γ-linolenic acids may be a function of the relative affinity of these acids for the polydehydrogenase and degradation systems, the latter removing active acetate which would be used for chain-lengthening.

It is possible, of course, that the ultimate true explanation for the transformations of the polyunsaturated acids will rest on an entirely different basis. However, at present, the proposed system serves to explain existing data on a logical basis and to predict results leading to further experimental verification. Both of these qualities are properties of a useful hypothesis.

SUMMARY

The formation and transformation of the polyunsaturated fatty acids in the animal body have been shown to be part of an orderly system similar to known reactions of saturated and monounsaturated acids. The results have led to a theoretical consideration of the mechanisms involved.

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