Interactions in the metabolism of polyunsaturated fatty acids: analysis by a simple mathematical model

TOM LINDSTROM and IAN J. TINSLEY

Departments of Mathematics and Agricultural Chemistry, Oregon State University, Corvallis, Oregon

ABSTRACT Interactions in the metabolism of polyunsaturated fatty acids have been simulated in a simple model system. In the development of this system it was assumed that simple competitive inhibition occurs between parent acids as they are transformed (via dehydrogenation and chain lengthening) to their derivative acids. Numerical solutions of this model system give the composition of the tissue pool of polyunsaturated acids as a function of the proportion of the parent acids in the diet. Experimental data have been analyzed in the light of relations generated by the model system and the parallels observed substantiate the assumptions postulated in the development of the model system.

KEY WORDS polyunsaturated fatty acids · metabolism · conversions · mathematical model · enzyme kinetics · competitive inhibition

THE POLYUNSATURATED fatty acids (PUFA) of mammalian tissues are derived primarily from the parent acids oleic, linoleic, and linolenic acids and to a lesser extent palmitoleic acid. Each parent acid gives rise to a series of PUFA characterized by the length of the saturated chain up to the first double bond in the molecule (counting from the methyl end). Most of the PUFA in tissue exist as complex lipids which show a degree of specificity in the identity of the fatty acids esterified at the α- and β-positions of the glycerol moiety. Thus the PUFA composition of a particular tissue must be determined primarily by the composition of the diet, the activity of the synthetic processes, and the requirements imposed in the synthesis of complex lipids.

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Abbreviation: PUFA, polyunsaturated fatty acids.

Recently it has been shown that rats fed increasing levels of linolenic acid build up in the liver higher proportions of the PUFA derived from this parent acid and at the same time the proportions of the PUFA derived from oleic and linoleic acids are reduced (1). Similarly, dietary linoleic acid can depress the proportions of PUFA derived from linolenic and oleic acids (2), and oleic acid the PUFA derived from linoleic acid (3, 4). This response is not simply due to dilution. One parent acid or its derivatives appears to intervene in the metabolism of the other series of PUFA.

This type of interaction might be explained by a competitive inhibition in the synthesis of various PUFA. Parent acids could react in the same synthetic system or inhibit the transformations in similar but different systems. Interactions could also occur in the synthesis of the complex lipids.

An explanation of these interactons will depend ultimately on the description of the appropriate synthetic pathways and an understanding of how the different fatty acids react in these systems. It is also possible to obtain some understanding of these interactions by interpreting present information in the light of a model system. Such an approach is outlined in this paper. It has been assumed that these interactions are due to competitive inhibition in the synthesis of PUFA. A simple model has been developed to simulate such a system and its response to different variables has been studied and related to available data.

DEVELOPING THE MODEL SYSTEM

Upon ingestion an unsaturated fatty acid is absorbed into the circulatory system and transported to the tissues. In the liver this acid can enter into synthetic pathways

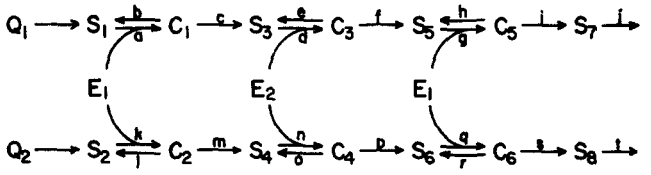

FIG. 1. A simple model showing competitive interactions in the synthesis of PUFA. Unsaturated fatty acids in the diet, Q_1 and Q_2, give rise to the same acids in the tissue S_1 and S_2. The latter are converted to PUFA by enzymes E_1 and E_2, via a series of enzyme–substrate complexes C_1, C_3, C_5 (or C_2, C_4, C_6) formed in reversible reactions and dissociating in irreversible reactions, all with rate constants denoted by lower case letters.

and be transformed into a series of higher homologues. One could thus postulate the existence of a pool of fatty acids that would be available for the synthesis of complex lipids. The fatty acid composition of the latter would reflect the composition of this pool with some modification due to specific fatty acid requirements in the synthesis of the complex lipids. We have attempted to simulate only the synthesis of PUFA, not considering the synthesis of complex lipids at this time.

The model system is described in Fig. 1; upper case letters represent the reacting components. In the mathematical treatment of the system (below), these symbols are used to represent actual values for the amounts of fatty acids put in to the system and the amounts of the tissue components. Rate constants for the individual reactions are denoted by the lower case letters.

An animal ingesting a ration of constant composition over a period of weeks could be considered to have a constant rate of intake of dietary components. Thus, Q_1 and Q_2 represent a constant input of two parent unsaturated fatty acids, which causes the appearance, at a constant rate, of the same acids, S_1 and S_2, in the tissue pool. The complex phenomenon of lipid absorption and transport is assumed to have first-order kinetics, i.e., we assume a simple proportional relation between the dietary supply and the amount in the tissue pool.

By a sequence of enzymatic reactions incorporating the appropriate enzyme-substrate complexes represented by C_1 to C_6, S_1 is converted to S_7 and S_2 to S_8. Since S_7 and S_8 may be converted further to other derivative acids, loss terms are included which give an open system. It has been assumed that the system is irreversible. The validity of this assumption might be questioned in the light of recent evidence that demonstrated the conversion of a 22:5 acid to arachidonic acid (5), but it is not known if this transformation is effected by the reversal of the synthetic process or by some other mechanism(s).

PUFA are synthesized from the parent acids by a combination of dehydrogenation and chain-lengthening steps. The mechanism of these processes has not been elucidated but it is probable that each process requires several steps. Thus E_1 and E_2 would represent a limiting enzyme in the dehydrogenation and chain-lengthening process respectively. It is assumed that the cofactors required for these reactions are not limiting. A simple competitive interaction results from the reaction of substrates from both sequences, $S_1 \rightarrow S_7$ and $S_2 \rightarrow S_8$, with enzymes E_1 and E_2.

Two dehydrogenation steps are required in this reaction sequence and it has been assumed that both are catalyzed by the same enzyme, E_1. Additional experimental evidence on the mechanism of this process is required to determine the validity of this assumption.

NUMERICAL SOLUTION AND RESPONSE TO INPUT VARIATIONS

Numerical solutions of this system which give values for the amounts of S_{1-8} and C_{1-6} as functions of time have been obtained by means of the procedure outlined in the Appendix. Kinetic constants have been chosen that give relative values of S_{1-8} and $Q_{1,2}$ which are comparable to those observed experimentally (4) if we take Q_1 to represent oleic acid and Q_2 linoleic acid.

In the transformation of linoleic acid to arachidonic acid the intermediates, 18:3ω6[1] (S_4) and 20:3ω6 (S_6), are present in only small amounts. Also the level of arachidonic acid is usually greater than that of linoleic acid, its precursor. A similar situation exists in the oleic acid series except that the principal derivative acid, 20:3ω9 (S_7), is present in smaller quantities than its parent acid. The kinetic constants provide solutions which reflect these general relationships. One thus makes the assumption that these relationships depend on the synthesis of the PUFA, not on the synthesis of complex lipids.

A solution of this system in which the arbitrary constants have been used is summarized in Fig. 2 where S_1, S_2, S_7, and S_8 are plotted as a function of time for input levels $Q_1 = 0.50$ and $Q_2 = 0.20$. Initial levels of S_{1-8} were considered to be zero. Since the rate constants are arbitrary, the time scale is also arbitrary. A steady-state system is generated that would correspond to the composition of the pool of PUFA of a tissue from an animal raised on a ration of constant composition. The data of Brenner and Nervi (6) indicate that a period of about 10 days is required to establish such a steady state in rat liver.

The significant aspect of this system is the manner in which the steady-state levels of S_{1-8} are influenced by variations in the relative amounts of Q_1 and Q_2. Most of the experimental data now available are the result of

[1] In this notation the PUFA are denoted by the number of carbon atoms in the chain, 18; the number of double bonds in the molecule, 3; and the position of the double bond counting from the methyl or "ω" end of the chain.

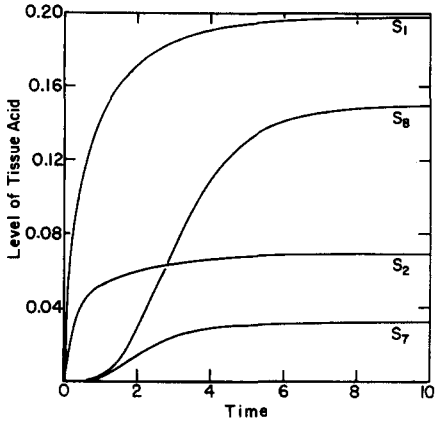

FIG. 2. Numerical solution of the model system for $Q_1 = 0.50$ and $Q_2 = 0.20$. Curves for four of the acids are shown.

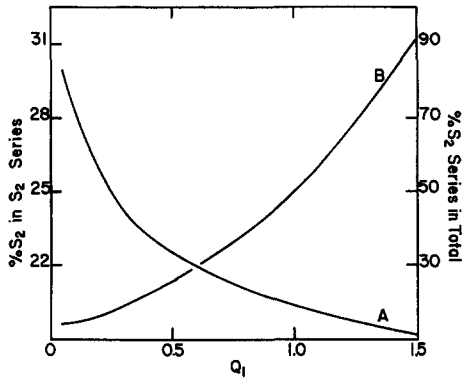

FIG. 3. The influence of Q_1 on the proportion of S_2 series acids in the total (*A*) and on the proportion of S_2 in the S_2 series of acids (*B*).

examination of this kind of variation, i.e., the relation between tissue and dietary fatty acid composition. A summary of steady-state levels of S_{1-8} for varying levels of Q_1 and Q_2 is given in Table 1.

If the value of Q_2 is held constant and the value of Q_1 increased, there is an obvious increase in the amount of the Q_1 series of PUFA. In addition this change in Q_1 results in a "backing up" in the transformation of S_2 to S_8. As Q_1 increases, one observes that the proportion of pool PUFA due to the Q_2 series decreases but in contrast the proportion of S_2 in the latter increases (Fig. 3). This effect is the direct result of the competitive situation and is also illustrated by a decrease in the S_8/S_2 ratio with increasing Q_1. S_6 also reacts with enzyme E_1 and responds in a similar fashion to S_2 with change in Q_1. A similar response is obtained in the Q_1 series with increasing increments of Q_2.

It is also noted that the S_8/S_2 ratio decreases with increasing Q_2 and the S_7/S_1 ratio decreases with increasing Q_1. This may result from the fact that both S_2 and S_6 or S_1 and S_5 react on the same enzyme. On the other hand it may simply reflect the normal relation between enzyme activity and substrate level as the enzyme level becomes limiting.

In reviewing the experimental data concerning the interactions of parent acids in PUFA metabolism one observes relations very similar to those generated by the model system. The "backing up" effect is well illustrated by the data of Rahm and Holman (2), who were studying the linolenate–linoleate interaction. Fig. 4 is derived from their data and one observes that as the level of dietary linoleate increases the proportion of the total fatty acids attributed to the linolenate ($\omega 3$) series decreases. At the same time the parent acid of this series, linolenic acid, represents an increasing proportion of the $\omega 3$ series of acids. Even though the dietary level of linoleate was increased by a factor of 60 the concentration of linolenate in the total fatty acids is essentially constant. The similarity between experimental data (Fig. 4) and the model system (Fig. 3) is quite apparent.

Mohrhauer and Holman (1) have demonstrated that increasing proportions of dietary linolenate decrease product–precursor ratios in the linoleic series of PUFA. A similar analysis of the fatty acid composition of liver lipids of rats fed rations containing high proportions of oleic acid indicates a comparable relation between oleic acid and the linoleic series of acids and linoleic acid and the oleic series of acids (3, 4). These data correspond to the relations between Q_1 and S_8/S_2 and Q_2 and S_7/S_1 summarized in Table 1.

When rats are fed rations containing adequate amounts of linoleic acid, the intermediate $20:3\omega 6$ is present in only small amounts in rat liver. However, we have observed that the proportion of this intermediate increases as that of $20:3\omega 9$ is increased by the feeding of fat rich in oleic acid (7). This response could also be related to the "backing up" effect suggested by the model system. Increasing levels of Q_1 increase the level of S_6 (Table 1).

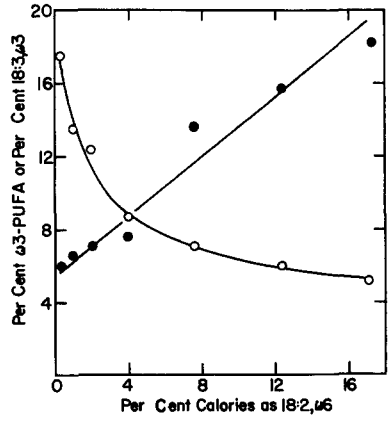

FIG. 4. Influence of dietary linoleate on the percentage of linolenate-derived ($\omega 3$) PUFA (—○—) in the total fatty acids and on the percentage of linolenate (—●—) in the $\omega 3$-PUFA. Calculated from the data of Rahm and Holman (2).

TABLE 1 STEADY-STATE VALUES FOR S_{1-8} AS Q_1 AND Q_2 ARE VARIED

Q_1	Q_2	S_1	S_2	S_3	S_4	S_5	S_6	S_7	S_8	S_8/S_2	S_7/S_1
0.05	0.10	0.015	.026	.007	.016	.002	.014	.003	.067	2.576	.200
0.10	0.10	0.030	.027	.014	.016	.003	.025	.006	.070	2.592	.200
0.20	0.10	0.063	.028	.029	.016	.007	.026	.012	.070	2.500	.190
0.50	0.10	0.188	.033	.075	.017	.019	.031	.030	.073	2.212	.160
1.50	0.10	1.311	.077	.255	.020	.133	.070	.103	.080	1.038	.079
0.05	0.20	0.015	.054	.007	.033	.002	.050	.003	.140	2.593	.200
0.10	0.20	0.031	.055	.014	.033	.003	.051	.006	.141	2.564	.194
0.20	0.20	0.066	.058	.029	.033	.006	.054	.012	.143	2.466	.182
0.50	0.20	0.197	.069	.076	.035	.020	.064	.031	.149	2.159	.157
1.50	0.20	1.432	.167	.260	.040	.145	.151	.105	.162	0.270	.073
1.0	0.02	.508	.008	.157	.003	.052	.008	.064	.015	1.875	.126
1.0	0.05	.518	.022	.158	.009	.053	.021	.064	.038	1.727	.124
1.0	0.10	.535	.046	.160	.018	.054	.043	.065	.078	1.695	.102
1.0	0.20	.572	.100	.164	.037	.058	.093	.067	.159	1.590	.117
1.0	0.50	.716	.313	.177	.102	.073	.288	.072	.424	1.354	.101

If rats are raised on a fat-free ration the steady state attained in liver tissue is characterized by a high proportion of 20:3ω9 and a low proportion of 20:4ω6. Brenner and Nervi (6) have fed linoleate supplements to rats which had been maintained on fat-free rations and observed the rate of change of the composition of liver fatty acids. The proportion of oleic acid and its derivative acid 20:3ω9 decreased in an exponential fashion to a lower steady-state level. After a lag period, attributed to the transformation of linoleic to arachidonic acid, the proportion of the latter increased to a new steady state. There was some indication that the proportion of linoleic acid rose to a maximum before dropping slightly to its new constant level. In the experiment the new steady state was achieved in 10–12 days.

This type of experiment has been simulated by obtaining a steady-state situation for $Q_1 = 1.00$ and $Q_2 = 0.001$. This would be equivalent to a fat-free diet with Q_1 representing an endogenous source of oleic acid. The steady-state values for S_{1-8} are used as initial levels and a solution has been obtained for the change of Q_2 to 2.00. This would represent a large increase in linoleate supply. The rate of change of the composition of the fatty acid pool is illustrated in Fig. 5. Values for S_1, S_2, S_7, and S_8 are plotted as a percentage of the total unsaturated fatty acids on the assumption that the model accounts for the total. The general relations depicted in this figure bear a striking resemblance to those obtained experimentally (6). Of interest is the lag in the buildup of S_8 and a maximum in the proportion of S_2. Both the experimental and simulated systems generate a new steady state.

This simple system has illustrated the type of relations one might expect in competing synthetic sequences in an open system. The parallel between the relations generated from numerical solutions of this model and experimental data constitute good evidence that the interactions in PUFA metabolism are of the type depicted by the model.

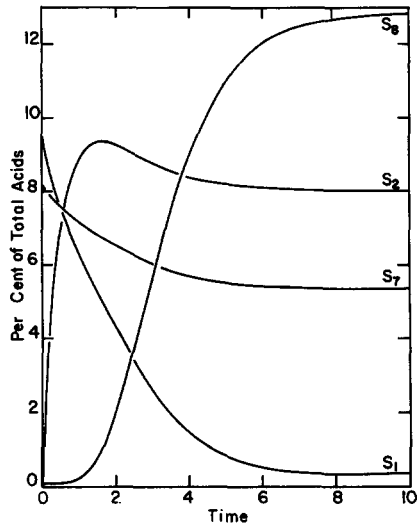

FIG. 5. The attainment of a new steady state when Q_2 is changed from 0.001 to 0.20; Q = 1.00.

Further studies should allow the assignment of real constants in such a system as well as its extension to include the synthesis of complex lipids and the incorporation of some of the requirements of these proceses. Thus the progressive development of such a system should give a more comprehensive simulation of the total biochemical system.

APPENDIX

The derivation and treatment of equations that were used to obtain numerical solutions of the system depicted in Fig. 1 are as follows. With the symbols as defined in the legend to Fig. 1, equations defining the rate of change of S_1, C_1, and E_1 (these symbols now representing amounts) can be written as follows:

$$dS_1/dt = -aE_1S_1 + bC_1 + Q_1$$

$$dC_1/dt = aE_1S_1 - (b + c)C_1$$

$$dE_2/dt = -dE_2S_3 - nE_2S_4 + (o + p)C_4 + (f + e)C_3.$$

The complete system is defined by writing comparable equations for the other reacting components. The enzymes E_1 and E_2 are considered to be true catalysts and the following conservation expression can be written:

$$E_1 + C_1 + C_2 + C_5 + C_6 = E_{t1}$$

$$E_2 + C_3 + C_4 = E_{t2}.$$

E_1 and E_2 represent the uncomplexed enzyme whereas E_{t1} and E_{t2} the total level of the enzymes in the system.

The kinetic equations listed above are the nonlinear type, with constant coefficients. No solution of these equations in terms of elementary functions is available.

One may obtain a steady-state solution by setting the derivatives equal to zero and performing the necessary algebra. S_{1-8} and C_{1-6} can then be expressed in terms of the input functions and the rate constants. Alternatively, a time solution may be obtained and the steady-state conditions derived from extended time values.

Numerical solutions of this system have been obtained using the process of continuous analytic continuation (8) with the stipulation that the imaginary part of the complex variable, Z, vanishes identically [Im $(Z) \equiv 0$]. The process then reduces to a successive approximation. The appropriate derivatives are computed from the original equations and incorporated in the approximating polynomials

$$S_p)_{i+1} = S_p)_i + \mathring{S}_p)_i(\Delta t) + \mathring{\mathring{S}}_p)_i\frac{(\Delta t)^2}{2!} + \dddot{S}_p)_i\frac{(\Delta t)^3}{3!}$$

$$S_q)_{i+1} = S_q)_i + \mathring{S}_q)_i(\Delta t) + \mathring{\mathring{S}}_q)_i\frac{(\Delta t)^2}{2!} + S_q)_i\frac{(\Delta t)^3}{3!}.$$

In this system p can have values 1, 3, 5, and 7 and q 2, 4, 6, and 8, corresponding to the two synthetic sequences and giving a total of eight equations. The subscript i takes integer values in the range $0 \leqslant i \leqslant M$ where M is the number of increments in the iterative process. A comparable set of equations is used for the derivation of C_{1-6}, the enzyme–substrate complexes. For a set value of Δt and given initial values of S_{1-8} and C_{1-6} this iterative process generates time solutions of all components of the system.

The error incurred using the process is given by the following relation:

$$\text{error} \leqslant (S_i^n(\xi)(\Delta t)^n M)/n!,$$

where n is the number of terms in the Taylor series used and Δt the fixed time increment, and ξ corresponds to the value of the nth derivative at some interior point t_i to t_{i+1}.

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