A Model for the Kinetics of Pyruvate Metabolism *in Vivo**

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ABSTRACT: Mice received radioactive label by intravenous injection, and were killed at different times. The fraction of radioactivity incorporated into circulating glucose is 6% for [2-^{14}C]acetate, 10% for [3-^{14}C]pyruvate, and 34% for [3-^{14}C]-malate. The fraction of the total fatty acids radioactivity which is randomized on all carbons of the chain increases with time, and reaches 9, 50, and 95% when the precursor is acetate, pyruvate, and malate, respectively. A scheme of biochemical interconversions is proposed, and all the data are interpreted with the help of a model which must be as simple as possible and must use the set of minimum parameters able to fit the experimental data. It is demonstrated that the radioactive homologs of components in steady state are converted by first-order reactions, which are represented by linear differential equations. The results of the calculations concern the whole organism. (1) Less than one-fifth of the pyruvate is decarboxylated to acetate and about half is carboxylated, the carboxylation proceeding by two different pathways of equal importance. (2) The equilibration between malic, oxaloacetic, fumaric, and pyruvic acid is achieved in a few seconds. About 60% of the mixture of the three former components is transformed into glucose, and 40% into pyruvate. (3) Approximately 73% of the acetate disappears, 20% is converted into malate, and 7% into fatty acids. (4) Gluconeogenesis from malate supplies at least 22.5% of the circulating glucose. (5) The half-lives of the metabolites under consideration cannot exceed a few seconds, except for glucose. The study was performed on a population of mice, and in spite of large individual variations, the solution is statistically significant. The significance of these abstract results is discussed.

Although pyruvic acid is an important intermediate in the metabolism of vertebrates and has been investigated by a number of authors, the information available concerning the quantitative aspect of its reactions *in vivo* is very poor, because the number of reactions involved makes the calculations uncertain. The investigator has to decide whether to use purified preparations, in which case the kinetics are simplified but do not correspond to the living reality, or to use the whole living organism, when the kinetics are real but too complicated.

However, we have adopted the second approach and attempted to build a coherent picture of pyruvic acid metabolism from the data obtained and by using certain limiting simplifying hypotheses. It is not possible to obtain all the parameters of a series of reactions because too many steps are involved and not enough data are available. However, and this is the main point of this work, it is always possible to determine, for a given transformation, which orders of magnitude the kinetic parameters may have, and which they cannot.

It is possible to suppose that a reaction has only one step that is not reversible, and that its effect is not decreased by parallel or antagonistic reactions. This hypothesis corresponds to the limit case allowing the maximal efficiency of the conversion, and the rate calculated in these conditions may be only equal or lower than the real rates.

Our data concern the conversion of pyruvate to glucose, fatty acids, and symmetric dicarboxylic acids of the Krebs cycle. More information on these conversions are obtained by using precursors other than pyruvate and by referring to the results of other investigators (see below, constraints of the model).

The conversions involved in the present study are represented in the Figure 1. We have no data on other reactions of pyruvate and other metabolites, but if these reactions are not considered, a limit exists, which represents the optimal yield for the conversions under consideration. For these conversions, represented in the Figure 1, the rate constants that have been calculated are not real; but they are the smallest possible since the conditions are optimal, and it is of some interest to know these limits.

However, even for simplified representations of metabolism, the mathematical analysis is not without difficulties, and a general theory, as well as the justification of a kinetic model describing the Figure 1 were developed.

The purposes of the present paper are: (a) to propose an application of this general treatment to a system of biochemical interconversions; (b) to see whether the animals of a given population have some common features with respect to quantitative metabolism; (c) to obtain minimum parameters and maximum metabolite half-lives from limit hypotheses; (d) to have an approximate idea of the fate in the whole organism of some metabolites.

Material and Methods

Experimental Procedure. Mice weighing from 30 to 40 g received a so-called normal diet, *i.e.*, a NAFAG food for mice, which was given during the night before the experiment and then four times during 10 min, at, respectively, 7.75 hr, 5.75 hr, 3.75 hr, and 45 min before the injection of the precursor. Different groups received [2-^{14}C]acetate, [3-^{14}C]pyruvate, [3-^{14}C]malate, and [6-^{14}C]glucose in intravenous injection. Moreover, all mice received acetate-2-t in the same injection.

The mice were killed at different time intervals ranging from a few seconds to 1 hr after the precursor injection. An aliquot of blood from 0.5 to 1.0 ml was deproteinized by precipitation with $BaSO_4$ and centrifugated. The blood glucose specific radioactivity was measured either directly

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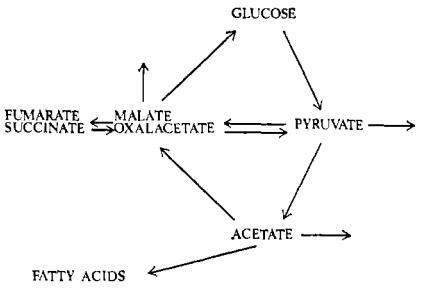

FIGURE 1: Scheme of the biochemical reactions involved in the present study. Explanations in the text.

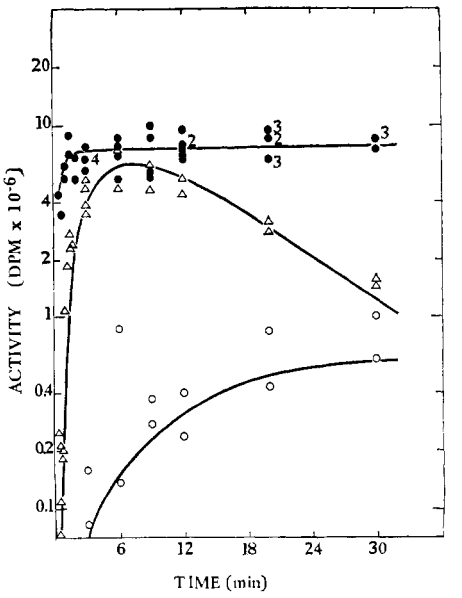

FIGURE 2: Radioactivity measured after injection of [2-^{14}C]acetate. (△) Total activity of circulating glucose. (●) Total activity of total fatty acids. (○) Randomized activity of total fatty acids. For all precursors, the fatty acids are those of the whole body except the liver and the blood, 10^6 dpm correspond to 1% of the label injected, and figure beside a dot gives the number of data points represented by this dot. The curves are those obtained by the statistical fit.

after absorption of ionized metabolites on Dowex 3 and Dowex 50W 8 resin (Corredor *et al.*, 1967) or as glucosazone (Feller and Strisower, 1950). The total glucose of this aliquot was measured by the *o*-toluidine method (Dubowski, 1962).

The fatty acids of the whole organism, except the liver and the blood, were extracted with petroleum ether (bp 30–60), purified, and their radioactivity measured. When necessary, the fatty acids were esterified into methyl esters, separated on Apiezon-L column at 245° by gas–liquid Autoprep Aerograph chromatograph, and the radioactivity of palmitic acid measured.

The palmitic acid was decarboxylated by the acid method (Schuerch and Huntress, 1949), the CO_2 gathered as $BaCO_3$, and its radioactivity measured by an ionization counter Tracerlab.

The other radioactivities were measured in a Packard Tri-Carb liquid scintillation spectrometer, in a 2,5-diphenyloxazole–1,4-bis[2-(5-phenyloxazolyl)]benzene–naphthalene–dioxane mixture for the resin-separated glucose, in a 2,5-diphenyloxazole–1,4-bis[2-(5-phenyloxazolyl)]benzene–toluene mixture for the fatty acids and osazones.

The quenching was determined according to Hendler (1964), the disintegrations per minute calculated by a FORTRAN IV program.

Calculations. It is necessary to transform our specific and total disintegrations per minute to utilizable data. Several works (Geissbühler and Favarger, 1965; Dupuis and Favarger, 1963; Handwerk and Favarger, 1959; Favarger and Gerlach, 1965) showed that the radioactivity of the palmitic acid biologically formed from a labeled precursor is equally divided between the eight two-carbon units of the chain. On the other hand, the precursors chosen in this work cannot give a carboxyl-labeled acetyl-CoA or palmitic acid, without randomization of the radioactivity on the two carbons of the acetyl group, or a two-carbon unit.

Therefore from the radioactivity of the palmitic acid carboxyl group the proportion of the total radioactivity which is randomized may be calculated and the balance is the so-called nonrandomized total activity.

For glucose, only the specific radioactivity may be measured. The specific activity of the whole blood glucose is a good approximation for the specific activity of plasma glucose (Baker *et al.*, 1959). The total radioactivity of the circulating glucose may be calculated by multiplying the specific radioactivity by the glucose pool size M_G, which is computed with the help of the SAAM 23 program. In the Figures 2–5, only the definitive total disintegrations per minute for the circulating glucose are considered.

In order to help the comparisons, each value corresponds to an injection of 45 μCi or 10^8 dpm. Therefore, 10^6 dpm in the results corresponds to 1% of the injected dose. As all the known conversions of L-malate are highly stereospecific, only the half of the DL-[3-^{14}C]malate injected was considered.

Very little correlation was observed between the weights of the mice and the measured radioactivity. Therefore, the results are not given for 100 g of the animal weight, but for every mouse measured.

Experimental Section

The results concerning the different precursors are as follows.

Acetate. The maximal radioactivity incorporated into the circulating glucose corresponds to 6% of the material injected, about 6–7 min after the injection. About 8% was found in the fatty acids, nine-tenths of this value being reached before 3 min. The randomization increases with time, up to 9% of the fatty acid radioactivity.

Pyruvate. A maximum of 10% of this label was found in glucose after 3–4 min. The label injected was found in fatty acids (3%), nine-tenths of this value being reached before 12 min. The randomization is 50%.

Glucose. The radioactivity injected is found in the fatty acids (2.5–3%), the nine-tenths of the maximal value is not yet reached until after 30 min.

Malate. A maximum of 34% of the radioactivity is found in the glucose after 6 min and 2.5–3% in the fatty acids, and as with the previous precursor the nine-tenths of the maximal value was not reached until after 30 min. The randomization is about 95%. These results are shown in Figures 2–5.

Theory

A living organism, notably a mammal, may be considered as a steady-state system in short-term experiments and in absence of hormonal or neuropsychologic stimulus. In such a

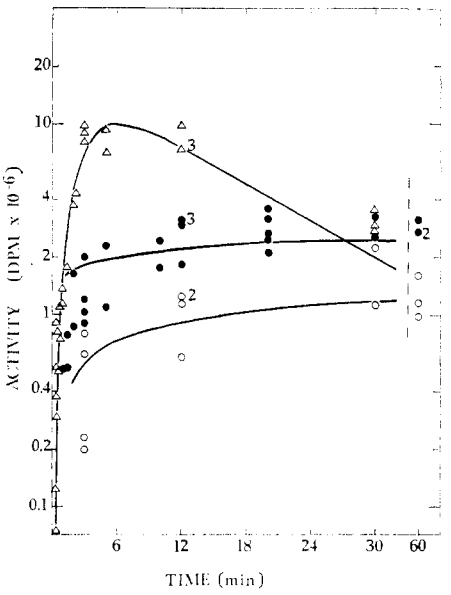

FIGURE 3: Radioactivity measured after injection of [3-^{14}C]pyruvate. (△) Total activity of circulating glucose. (●) Total activity of total fatty acids. (○) Randomized activity of total fatty acids. The curves are those obtained by the statistical fit.

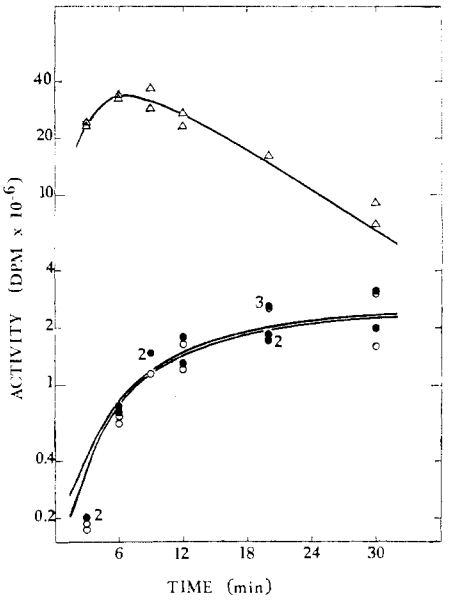

FIGURE 5: Radioactivity measured after injection of [3-^{14}C]malate. (△) Total activity of circulating glucose. (●) Total activity of total fatty acids. (○) Randomized activity of total fatty acids. The curves are those obtained by the statistical fit.

system, the calculations for a set of first-order reactions are carried out by a set of linear differential equations. It will be demonstrated that labeled metabolites coming from an unique injection of a radioactive substance are converted by first-order reactions only if their unlabeled homologs are in a steady state.

Equation for Unlabeled Metabolites. Let us examine the following reaction sequence in the steady state with a constant supply of A and B

$$\longrightarrow A + B \xrightarrow[k]{} P \xrightarrow[k_{oP}]{}$$

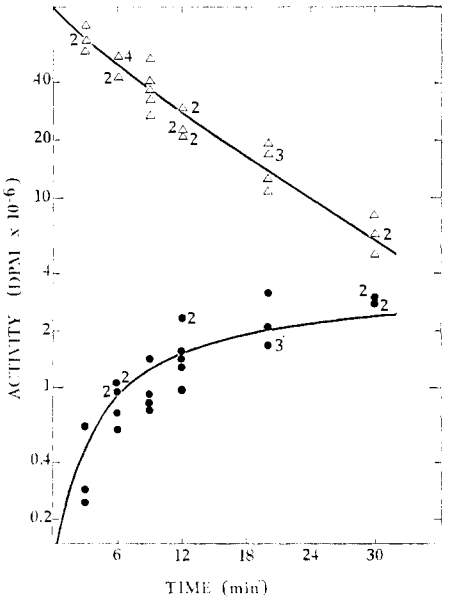

FIGURE 4: Radioactivity measured after injection of [6-^{14}C]glucose. (△) Total activity of circulating glucose. (●) Total activity of total fatty acids. The curves are those obtained by the statistical fit.

where k_{oP} is the rate constant of conversion of P into another substance not considered in the present study. According to the fundamental equations of chemical kinetics, we have the general relation

$$\frac{d[P]}{dt} = k[A]^a[B]^b - k_{oP}[P]^p = 0 \quad (1)$$

Under steady-state conditions, [A], [B], and [P] are constant. With the following definitions

$$\begin{aligned} \lambda_{PA} &= k[A]^{a-1}[B]^b \\ \lambda_{PB} &= k[A]^a[B]^{b-1} \\ \lambda_{oP} &= k_{oP}[P]^{p-1} \end{aligned} \quad (2)$$

eq 1 becomes

$$\frac{d[P]}{dt} = \lambda_{PA}[A] - \lambda_{oP}[P] = \lambda_{PB}[B] - \lambda_{oP}[P] = 0 \quad (3)$$

In the same way, if [C] is defined as the number of A and B units incorporated into P:

$$\frac{d[C]}{dt} = \lambda_{CA}[A] + \lambda_{CB}[B] - \lambda_{oC}[C] = 0 \quad (4)$$

The generalization for a system with n components is for $i = 1, 2, \ldots, n$

$$\frac{d[i]}{dt} = \sum_k^n \lambda_{ik}[k] = 0 \quad (5)$$

λ_{ik} = rate constant of the transformation of k into i. This set of n equations can be easily solved, since the differential members may be eliminated.

Equations for Labeled Metabolites. Every metabolite has a constant level, for the rate of its renewal equals the rate of its destruction. If a radioactive homolog i^* of a metabolite i is introduced into the system, or originates from another injected labeled substance, its level will vary with time. This problem has already been treated, but in a less general way (Robertson, 1957; Rescigno and Segre, 1961; Berman, 1968; Neiman and Gal, 1970). For any reaction A + B → P, we have

$$\frac{d[P]}{dt} = k[A]^a[B]^b = -\frac{d[A]}{dt} = -\frac{d[B]}{dt} \quad (6)$$

At each time interval between t and $t + dt$, the proportion of A and B which is transformed corresponds to the probability of destruction for every single A or B molecule. Therefore, during this time interval, this proportion is the same, for the homogeneous A molecule set as well as for a part A* of this set.

Therefore

$$\frac{d[A^*]}{[A^*]} = \frac{d[A]}{[A]}$$

and

$$\frac{d[B^*]}{[B^*]} = \frac{d[B]}{[B]} \quad (7)$$

If [A*] and [B*] are the concentrations of radioactive molecules and [C*] the concentration of the labeled units (and not of molecules) in the P molecule ensemble, the influence of [A*] and [B*] on [C*] is

$$\frac{d[C^*]}{dt} = -\frac{d[A^*]}{dt} - \frac{d[B^*]}{dt} \neq 0 \quad (8)$$

and by substitution by the value found in eq 7, then in eq 6

$$\frac{d[C^*]}{dt} = +\frac{[A^*]}{[A]}k[A]^a[B]^b + \frac{[B^*]}{[B]}k[A]^a[B]^b \quad (9)$$

By introducing the parameters defined in eq 2, eq 9 becomes

$$\frac{d[C^*]}{dt} = \lambda_{CA}[A^*] + \lambda_{CB}[B^*] \neq 0 \quad (10)$$

The state described by this relation is the transient state mentioned by Landau *et al.* (1964). [C*] is the concentration of the radioactive units in the P molecule ensemble. If this unit used in the calculation is not the molecule, but an "equivalent" chosen according to the needs, any single P molecule has as many equivalents C as it contains groups susceptible to be labeled. Reiners's objections are overcome (Reiner, 1953a,b) and the problem may be solved by linear algebra. Thus the existence of a steady state is a necessary and sufficient condition to have first-order reactions for the labeled metabolites, and to justify a treatment with a linear differential equations system. Such a conclusion has been confirmed experimentally (Neiman and Gal, 1970; Berezin *et al.*, 1957a,b). The steady state is not disturbed by injection of a low dose of highly radioactive precursors.

The eq 4 and 10 have the same form; λ_{CA} refers to both conversions of A into C and A* into C*, and λ_{CB} into those of B into C and B* into C*. The generalization for a system of n metabolites k is (for $i = 1, 2, ..., n$)

$$\frac{d[i^*]}{dt} = \sum_k^n \lambda_{ik}[k^*] \neq 0 \quad (11)$$

where λ_{ik} = conversion rate constant of k into i, and for the total activities X_i

$$\frac{dX_i}{dt} = \sum_k^n \lambda_{ik}X_k \neq 0 \quad (12)$$

The solution of such a system, for distinct roots, is in the form

$$X_i = \sum_j^n \alpha_{ij}e^{\beta_j t} \quad (13)$$

where α_{ij} and β_j are constants to be related to the λ_{ik}. The set of transformations is

$$\sum_i^n \frac{dX_i}{dt} = \sum_i^n \sum_k^n \lambda_{ik}X_k \quad (14)$$

or in matricial form

$$\left(\frac{dX_i}{dt}\right) = [\lambda_{ik}](X_k) \quad (15)$$

The initial conditions of each X_i are known, for they equal zero except for the injected substance. After every time unit, we have for each X_i

$$X_{i(t+\delta t)} = X_{i(t)} + \delta X_i \quad (16)$$

On this basis the λ_{ik} are approximated by a simulation method, and then fitted with the help of the SAAM 23 program (Berman *et al.*, 1962a,b).

The Model. The complexity of an organism makes it impossible to follow a metabolic sequence step by step and *a fortiori* the elementary steps of a single reaction in the sequence.

Therefore, the concept of mathematical pool is introduced. Any metabolism may have an abstract representation, which is the system described above or a compartment model. For this system, the steady-state hypothesis and the numerical values calculated for λ_{ik} parameters are valid only if each compartment k of the model contains a definite quantity M_k of material. This quantity M_k is called the mathematical pool of the substance k. The mathematical model need not define distinct chemical pools in precise subcellular or anatomical locations.

The constraints of the model are as follows. (1) It must be as simple as possible and must contain only the compartments referring to the metabolites injected or measured and a minimum number of intermediate compartments. These compartments are extended to the whole organism. The simplest hypothesis is the one whose treatment requires the lowest range of experimental data. This concept recalls chemical kinetics, in which a step is considered as elementary when no experimental data permit its division (Polak, 1969), because supplementary parameters would lead to trivial solutions. There-

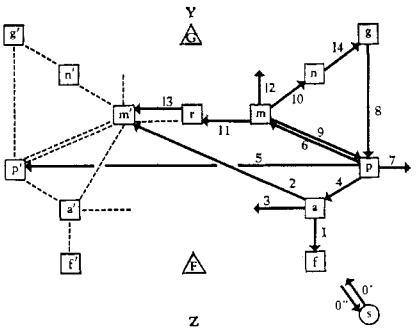

FIGURE 6: The model. (□) Basic compartments; (△) composed compartments; (○) injection compartment. a, . . . , j, . . . , nonrandomized compartments; a′, . . . j′, . . . , randomized compartments; A, . . . J, . . . , composed compartments. The compartments are: a, acetate or acetyl-CoA; f, fatty acids; g, glucose; m, malate + oxaloacetate; n, gluconeogenesis, p, pyruvate; r, randomization (in the Krebs cycle); s, injection. ←, computed parameters. ←┄, dependent parameters.

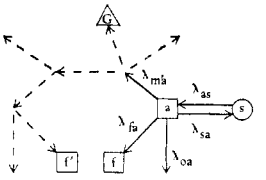

FIGURE 7: Scheme of the disappearance and scattering of injected labeled acetate. Only the channels represented by the full arrows share out the total radioactivity injected. The compartments are: a, acetate; f, nonrandomized fatty acids; f′, randomized fatty acids; G, circulating glucose; s, injection.

fore, any attempts of dividing the existing compartments are as arbitrary as the simplest hypothesis, even if it seems more justified by biological considerations. (2) The model must use the set of the minimum λ_{ik} parameters able to fit the experimental data. (3) The label injected is always nonrandomized. (4) The randomization of [2-^{14}C]acetate, [3-^{14}C]pyruvate, [6-^{14}C]glucose, and [3-^{14}C]malate occurs in pathways that involve a symmetric dicarboxylic acid of the Krebs cycle (for scheme of randomization, see Heath, 1968). Several other pathways were examined, but none is able to randomize significantly the label of our precursors in the fatty acids. (5) In the present state of knowledge, most authors admit that the following intermediates are obligatory: (a) acetyl-CoA between pyruvate and fatty acids; (b) oxaloacetate and malate, between pyruvate and glucose, between pyruvate and symmetric dicarboxylic acids of Krebs cycle, between these symmetric acids and fatty acids or glucose, between acetyl-CoA and glucose; and (c) pyruvate between glucose and fatty acids, between oxaloacetate or malate and fatty acids. (6) On the other hand, there is poor incorporation of radioactivity from [1,5-^{14}C]citric acid into fatty acids (Arbex *et al.*, 1970), and radioactivity from [2,3-^{14}C]succinate is incorporated 20 times more than that from [1,4-^{14}C]succinate (Favarger and Gerlach, 1961). (7) According to the constraint 2, the part of symmetric acids which is not converted to randomized malate is neglected. This assumption corresponds to the limit condition of best "yield" of the randomization, in other words we can only overestimate in the calculations the effect of interaction rate constants between symmetric acids and oxaloacetate or malate, and therefore underestimate these constants. (8) Since glucose and pyruvate radioactivities show about the same incorporation into fatty acids, no exit parameter was given to the glucose compartment. The direct oxidation of glucose seems low (Baker *et al.*, 1961). (9) The fate of the label depends also on its position in the molecule, and it is the label that is being followed. Therefore, every metabolite will be represented in the model by two compartments, randomized and nonrandomized, which are distinct only with respect to randomization, and not in the ordinary sense. The λ coefficients are also paired, both coefficients of a pair are linked by a definite relation, which depends only on the position of the label. For the conversions under consideration here, this relation is the identity, because the fate of randomized and nonrandomized label may be considered as the same, the only difference are the data we have on both randomized and nonrandomized fatty acids compartments. The randomization is complete after the first conversion into symmetric acids. Several turns in the Krebs cycle may only lead to waste of randomized label (Freedman and Graff, 1958), and do not influence calculations which are made on the limit hypothesis of best "yield" of randomization. Rather complicated mathematics are thus avoided (Weinman *et al.*, 1957).

The model represented in Figure 6 has an axis of symmetry *YZ* which bears a so-called randomization compartment *r*. This compartment may undergo exchanges only with randomized compartments, for a substance "already interconverted with symmetric acids" is not able to become a substance "not yet interconverted with symmetric acids." Moreover, the acetate radioactivity is randomized by the conversion of acetate into malate *via* the Krebs cycle, and both acetate compartments flow into the randomized malate compartment. These parts of the model are assymmetric in respect with the axis. Some results concern the total fatty acids, or the total glucose the randomized and nonrandomized ones being considered as a whole, and are expressed in a so-called "composed" compartment (Berman, 1968).

The comparison between the radioactivity curves of blood glucose, due to different labels injected, allows the supposition of a limiting step between malate and glucose. If this curve reaches a maximum at a time t_m and then decreases, we can picture it as a "radioactivity peak" occurring at the time t_m. Berman (1968) showed that the more steps there are in such a process, the narrower the peak. The form of the peak found here corresponds to a two-step conversion from malate to glucose and therefore two so-called "gluconeogenesis" compartments were introduced (*n* and its symmetric *n′* in the Figure 6). Any attempt of fitting without these compartments was unsuccessful.

Glucose radioactivity is about three times higher when labeled malate is injected, then when pyruvate is injected. For randomized fatty acids, the relation is only 1.7. Therefore, it was necessary to introduce a parameter $\lambda_{p'p}$, because this difference is not explainable if most part of pyruvate flows through malate compartment before reaching glucose or randomization compartments. This parameter is not inconsistent with the above hypotheses: it is permissible to suppose a flow from the p compartment to the p′ *via* the m*, r*, and m′* compartments, which have no exit toward glucose, and which are not mentioned because their lives are too short.

The final model has 16 compartments and 28 interconversion parameters, and the total number of data points is 245. The model itself leads to a segmentation of the problem, for which a unique solution is found. First, the two parameters connected to the injection compartment were computed in another work (P. Mermier, to be published).

TABLE I: Conversion Rate Constants, in Whole Normal Mice, Liver Excepted.

Symbol	Parameter	From	To	Av Value (sec^{-1})	Fractional Dev (%)
λ_{fa}, $\lambda_{f'a'}$	1	Acetate	Fatty acids	0.0027	20.1
$\lambda_{m'a}$, $\lambda_{m'a'}$	2	Acetate	Malate	0.007	20.6
λ_{oa}, $\lambda_{oa'}$	3	Acetate	Exit	0.027	21.5
λ_{ap}, $\lambda_{a'p'}$	4	Pyruvate	Acetate	0.036	65
$\lambda_{p'p}$	5	Pyruvate	Pyruvate	0.053	79
λ_{mp}, $\lambda_{m'p'}$	6	Pyruvate	Malate	0.042	63
λ_{op}, $\lambda_{op'}$	7	Pyruvate	Exit	0.076	65
λ_{pg}, $\lambda_{p'g'}$	8	Glucose	Pyruvate	0.0022	6.5
λ_{pm}, $\lambda_{p'm'}$	9	Malate	Pyruvate	Very rapid	
λ_{nm}, $\lambda_{n'm'}$	10	Malate	Gluconeogenesis	Very rapid	
λ_{rm}, $\lambda_{r\cdot m'}$	11	Malate	Randomization	Very rapid	
λ_{om}, $\lambda_{om'}$	12	Malate	Exit	Very rapid	
$\lambda_{m'r}$	13	Randomization	Malate	0.123	12.9
λ_{gn}, $\lambda_{g'n'}$	14	Gluconeogenesis	Glucose	0.021	29.5
$\lambda_{\cdot s}$	0·	Injection	Model	0.210	19.5
$\lambda_{s\cdot}$	0:	Model	Injection	0.026	55

Secondly, as shown above, all parameters are paired, except those of the flow going from the randomization compartment to the randomized malate compartment and from the nonrandomized to the randomized pyruvate compartment.

Third, as four precursors are used, the 245 data points are divided in four groups of results, which are represented by the Figures 2–5. For each group, acetate, pyruvate, glucose, and malate, the label injected flows from the injection compartment s to a precise nonrandomized compartment.

For instance, when the label is injected as acetate, the radioactive material flows from the injection compartment s to the "nonrandomized acetate" compartment a, and then flows further to the data compartments F, G, f, f′ or to exit of the system, *via* the λ_{fa}, $\lambda_{m'a}$, λ_{oa} parameters (Figure 7).

The numerical solutions of the equation system depend heavily on the λ_{fa}, $\lambda_{m'a}$, and λ_{oa} parameters, for the total radioactivity injected flows through the channels represented by these parameters, while the subsequent conversions act on much less labeled material. The data points of other groups have a small influence on the average value and on the standard deviation of these λ_{fa}, $\lambda_{m'a}$, and λ_{oa} parameters.

Therefore, partial computations with one group of data and parameters are made successively, until the same set of 28 parameters accounts for the four groups of results. The fact that we find stable solutions after a few iterations is the main justification of the model and the procedure used.

For the blood glucose radioactivity, the first measurements begin 10 sec after the injection of the label. As the latter flows through several compartments before reaching the glucose compartment, the kinetics can be estimated to one order of magnitude lower, *i.e.*, to a one second limit, but not more. For this reason, the upper limit of the parameters was arbitrarily fixed at 0.500 sec^{-1}. If a parameter reaches this upper limit, the compartment having this limit parameter is considered as a "very short-lived" compartment, and all of its exit parameters have undefined values.

After the computation of the λ parameters, the size of the pairs of compartments, randomized and nonrandomized taken as a whole, is calculated according to the relation 5.

The input in a steady-state system has to equal the output R which is

$$R = \lambda_{op}(M_p + M_{p'}) + \lambda_{oa}(M_a + M_{a'}) + \lambda_{om}(M_m + M_{m'}) \quad (17)$$

The input I_G into the glucose is arbitrarily proposed to represent the total system input I. The variation of the glucose level will be

$$\lambda_{gm}(M_m + M_{m'}) + I_G - \lambda_{pg}(M_g + M_{g'}) = 0 \quad (18)$$

The part N of gluconeogenesis from malate in the total glucose renewal is given by the relation

$$N = \frac{\lambda_{gm\cdot}(M_m)}{\lambda_{gm\cdot}(M_m) + I_G} \quad (19)$$

Since I_G may be only lower than I, and therefore is always overestimated, N cannot be higher than the real part.

Results and Justification. The values of the parameters are given in Table I, the relative importance of the conversions in Figure 8 and the ideal metabolite levels and conversion rates in Figure 9.

The exit parameters of malate are considered as being very high. They are undetermined, because their standard deviation is higher than their average value. This feature is understandable, for their influence on the results is masked by parameters of lower value. The λ_{om} and $\lambda_{om'}$ parameters may be considered as equal to zero.

Less than one-fifth of the pyruvate is decarboxylated to acetate, a third has an unknown fate, about half is carboxylated. This carboxylation itself proceeds by two different pathways of approximate equal importance (Figures 8 and 9), the interpretation of which will be discussed. The equilibration between malic, oxaloacetic, fumaric, and pyruvic acid is achieved in a few seconds, three-fifths of the mixture of the

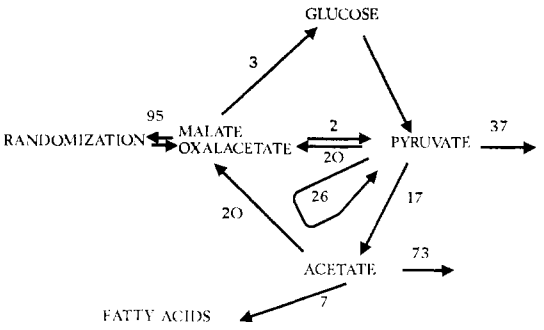

FIGURE 8: Fate of the metabolites. The figures on the arrows give the per cent of the metabolite converted *via* the pathway represented by the arrow.

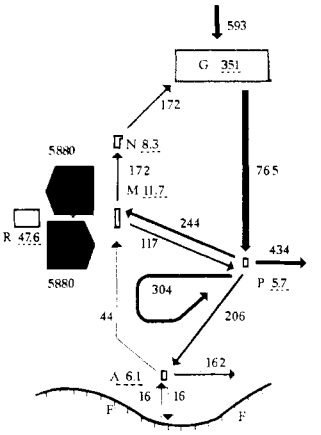

FIGURE 9: Ideal quantities and conversion rates of metabolites for a whole organism. 351, quantity of a metabolite, in microequivalents; 593, conversion rate of metabolite, in nanoequivalents. We define here an equivalent as the quantity of two- or three-carbon units corresponding to 1-g unit; for instance, 1 mole of glucose has 2 equiv, 1 mole of palmitic acid 8. A, Acetate or acetyl-CoA; F, fatty acids; G, glucose; M, malate; N, neoglucogenesis intermediates; P, pyruvate; R, randomization intermediates.

three former components is transformed into glucose, two-fifths into pyruvate.

Three-quarters of the acetate disappears, most part of this as CO_2 (P. Mermier, to be published), one fifth reaches the malate *via* the citrate and 7% the fatty acids.

The maximal compartment half-lives (τ) and renewal times (T)[1] are, respectively, acetate, 19 and 27.5 sec; pyruvate, 3.3 and 4.8 sec; glucose, 5 min, 18 sec and 7 min, 40 sec; malate oxaloacetate, 1 and 2 sec; gluconeogenesis, 33 and 48 sec; randomization, 5.8 and 8 sec.

At least the 22.5% of the input into the glucose compartment are furnished by the malate.

For each group, the correlation coefficients between the parameters, as well as the number of degree of freedom, are high, and the hypothesis of noncorrelation may be disregarded.

Discussion

All data have the same statistical weight, for the absolute errors were nearly the same for each measurement.

The good fit itself shows that in spite of large individual variations, the metabolism follows a general line, also from the quantitative point of view and that this line is valid not only for a whole organism, but also for a group of animals if the conditions are the same.

Many models can probably be consistent with the results, the range of which is large but there is only one simple model. For this model the uniqueness of the solution cannot be proved, but the correlation between the exit parameters of each compartment is too strong to allow any change without large consequences. In other models proposed, one or two parameters were somewhat lower, but in order to lower them, other parameters had to be increased in much larger proportions. Therefore, we have really the minimum order of magnitude for each parameter.

These results are abstract values, and in moving from abstract concepts to realities, the hypotheses governing the theoretical treatment must be kept in mind. Thus the meaning of the glucose and gluconeogenesis compartments are linked because the form of the curve of glucose radioactivity corresponds to a two-step conversion from malate to glucose. The following dilemma exists: either there are one or two limiting steps in the conversion of malate into blood glucose, or the glucose compartment contains, besides glucose itself, other substances in rapid equilibration with it. A resultant of these extremes is not excluded, for instance if the metabolites in equilibria are precisely the intermediates of the gluconeogenesis.

The part of the input into the glucose compartment coming from malate cannot be lower than 22.5% but is perhaps much more important. The lower limit is twice as high as Holt's *et al.* results (1961a,b) on the living rat and corresponds to Bergman's *et al.* findings (1966) in sheep. For Katz and Dunn (1967) about one-third of the ^{14}C derived from glucose is recycled in the living rat.

The other origins of glucose are not considered in this study, and may be food carbohydrates. Since glucose and pyruvate show about the same incorporation into fatty acids, the glucose compartment practically flows only into the pyruvate compartment, and there remain two possibilities for the exchanges between glucose and a part of glycogen. They may be very fast, and this part of glycogen belongs to glucose pool, or these exchanges cannot represent more than a few per cent of the input into the glucose compartment. The latter statement is likely as long as the animal is in a resting steady state.

The calculated volume of the glucose compartment is about half of the whole body volume of the animal. Since only 20% of the body weight is contributed by the extracellular fluid (Winters *et al.*, 1969), part of the intracellular glucose is rapidly equilibrated with blood glucose.

We have no data on the randomization of [2-^{14}C]- or [3-^{14}C]-pyruvate in blood glucose, but according to the model, it *would* reach 95%. This value is consistent with the results of many authors (Hoberman and D'Adamo, 1960; Lorber *et al.*, 1950; Topper and Hastings, 1949; Landau *et al.*, 1955; Hostetler *et al.*, 1969).

The question of the equilibration between malic and fumaric acids is still discussed. This equilibration is almost complete in the present work as shown by the importance of the randomization of malate label. For Heath and Threlfall (1968), it is far from complete in the liver *in vivo*. *In vitro*,

[1] τ_i is well known and given by the relation: $\tau_i = \ln 2/\sum_k \lambda_{ik}$. According to Zilversmit (1943), T is the time required for the appearance or disappearance of an amount of a substance i equal to the amount of that substance present in the system. In contrast to Reiner's demonstration (1953a,b), $\tau_i \neq T_i = 1/\sum_k \lambda_{ik}$, since the present system is open and dynamic steady state.

labels of malate and aspartate are well symmetrized in rat liver mitochondria (Walter *et al.*, 1966). *In vivo*, the distribution in glutamate of label injected as [2-^{14}C]alanine shows that the radioactivity is incorporated into citrate *via* AcCoA, and *via* oxaloacetate (Freedman and Graff, 1958; Koeppe *et al.*, 1959), before equilibration with fumarate (Freedman *et al.*, 1960). As almost all citrate runs the whole cycle (Heath and Threlfall, 1968), part of the randomization may be achieved by these two ways, the former included in the parameter $\lambda_{m'a}$, the latter in $\lambda_{r'm}$.

The computation showed that there are two ways by which randomization of the pyruvate label occurs *via* malate. It is clear that we do not believe that these ways correspond to two morphologic units, the first transforming the malate to glucose and fatty acids, the second only to fatty acids. The living organism contains a number of sites where malate, oxaloacetate, and metabolically similar molecules are converted at different proportions for each site into glucose, or into fatty acids *via* pyruvate. The model only means that the label injected as pyruvate prefers sites which are more oriented toward fatty acids (and less toward glucose metabolism) than the label injected as malate. Moreover, the kinetics are quicker where the conversions of malate into pyruvate are comparatively more important.

The fit overestimates the speed of the conversion of pyruvate into acetyl-CoA. This overestimation is due to the strong correlation between the λ_{ap} and the λ_{mp} parameters, the latter being very high. However, the difference between the data and the theoretical curve is not important enough to justify the insertion of an intermediate compartment between the pyruvate and the acetate compartment.

That means that the pyruvate compartment is also a set of metabolic sites, part of them having more exchanges with malate have also more rapid kinetics. This statement agrees with the above comments on malate compartment. Perhaps part of the λ_{op} corresponds to [3-^{14}C]pyruvate label which enters in citrate *via* oxaloacetate and is lost in the following steps of Krebs cycle (Rognstadt, 1969).

The sites which are more oriented toward oxidative decarboxylation have a slower activity. The question arises whether there is a limiting step in the decarboxylation or in the transport of substrate, or competition of highly reversible conversion into lactate, alanine, or formation of intramitochondrial acetyl-CoA and transport into cytoplasm, etc. The present state of knowledge does not allow any answer.

The concept of an "acetate" compartment extended to the whole organism does not exclude the existence of many metabolic centers where in addition to acetate and acetyl-CoA, other metabolites are involved. In our model, the flow from the acetate compartment to the fatty acids compartment is comparatively a slow process, and could be due to the conversion of acetate to acetyl-CoA (Sauer *et al.*, 1970), or to a limiting step controlled by the acetyl-CoA carboxylase. In the former case, the kinetics of the intermediate between pyruvate and fatty acids were faster than in the model, and the rate of pyruvate oxidation lower, but not the exit parameter from pyruvate, because the fit would then require the insertion of an additional compartment between the "pyruvate" and "acetate" compartments. In the latter case, a limiting step in the carboxylation of acetyl-CoA to malonyl-CoA would reduce both significations of acetate activation and pyruvate oxidation. The data are consistent with the existence of these three limiting steps, as well as with the possibility of rates limited by inter and intracellular diffusions, but do not allow a computation of their respective importance.

The pyruvate compartment contains 5.72 μmoles for the whole body. According to the concentration known in the adipose tissue (Ballard and Hanson, 1969), the liver (Williamson *et al.*, 1967; Threlfall and Stoner, 1961; Heath and Threlfall, 1968), and the blood (Exton and Park, 1967), this compartment probably refers for its most part to lactate and alanine.

A comparison between several studies on adipose tissue allows us to calculate a half-life of 5 sec for pyruvate alone and 30 sec for the pyruvate–lactate pool (Flatt and Ball, 1964; Ballard and Hanson, 1969). In the liver, the half-lives of pyruvate and pyruvate–lactate pools are 5 and 50 sec, respectively (Heath and Threlfall, 1968), or according to Walter *et al.* (1966) and Threlfall and Stoner (1961), 0.4 and 16 sec.

Most parts of these compounds are renewed, since during 1 hr adipose tissue only uses 1% of the pyruvate or lactate added (Schmidt and Katz, 1969). It may be noticed that the half-lives of pyruvate–lactate pools calculated for single tissues are clearly larger than the half-life we found for a pyruvate compartment extended to the whole organism. The opposite would be expected: thus the apparent life of glucose is eight times shorter in the perfused rat liver (Exton and Park, 1967) than in the whole mouse (present work). This paradox reflects perhaps differences in experimental conditions and mathematical interpretation. Sauer *et al.* (1970) worked *in vivo*, but on rat liver, and used the same mathematical approach as we did, but found longer half-lives for the di- and tricarboxylic acids as well as for acetyl-CoA and pyruvate. The question arises whether the metabolism of these intermediates is slower in rats than in mice, or in liver than in many high-energy-requiring tissues, such as muscles and heart.

Our work allowed us to settle the maximal half-life of compartments extended to the whole body. Every compartment probably contains many metabolites in equilibrium; moreover, a great deal of time is "lost" by reversible reactions, histologic and anatomic translocations. Consequently, the actual metabolites corresponding to the compartments have half-lives lower than one second, we may not say how much. Already in the model of Reich *et al.* (1968), the intermediates of glycolysis have half-lives which do not exceed a few seconds.

The statement enhances the importance of the factors involved by the localization, since a substrate may be stopped at a given site only because it has a too slow diffusion and no alternative but to react with the enzyme located there. On the other hand, the sites of slower metabolism do not act on enough material to lower the parameters calculated for the whole body. These parameters represent the threshold of reaction rates which must be reached. The very height of this threshold may influence the understanding of biological phenomena.

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