The Tricarboxylic Acid Cycle in *Dictyostelium discoideum*

A MODEL OF THE CYCLE AT PRECULMINATION AND AGGREGATION

By Patrick J. KELLY, Joanne K. KELLEHER and Barbara E. WRIGHT

Department of Developmental Biology, Boston Biomedical Research Institute, 20 Staniford Street, Boston, MA 02114, U.S.A.

(Received 23 April 1979)

A preliminary model of tricarboxylic acid-cycle activity in *Dictyostelium discoideum* is presented. Specific-radioactivity labelling patterns of intra- and extra-mitochondrial pools are simulated by this model and compared with the experimental data. The model arrived at by this method shows the following features. (1) The cycle flux rate is approx. 0.4 mm/min. (2) Both fumarate and malate are compartmentalized at approx. 1:5 between cycle pools and non-cycle pools. These may represent mitochondrial and cytoplasmic pools. Citrate is compartmentalized at 1:10. Succinate appears to exist in three compartments, two of which become labelled by [^{14}C]glutamate and only one by [^{14}C]aspartate. (3) Two pools of aspartate with two associated pools of oxaloacetate are necessary for simulation. (4) Exchange between the cycle and non-cycle pools of both citrate and fumarate occurs at very low rates of about 0.0003 mm/min, whereas exchange between the malate pools is about 0.004 mm/min. The exchange reaction glutamate $\rightleftharpoons$ 2-oxoglutarate runs at approx. 15 times the cycle flux. (5) A reaction catalysed by 'malic' enzyme is included in the model, as this reaction is necessary for complete oxidation of amino acid substrates. (6) Calculation of the ATP yield from the model is consistent with earlier estimates of ATP turnover if the activity of adenylate kinase is considered.

Energy metabolism during differentiation and aging in *Dictyostelium discoideum* is characterized by a net degradation of cellular protein with the resultant amino acid residues functioning as substrates for tricarboxylic acid-cycle activity. That protein is the major energy source in this system is strongly supported by the demonstrated synthesis of acetyl-CoA from malate, catalysed by 'malic' enzyme (Kelleher *et al.*, 1979), and by the lack of significant gluconeogenesis and glycolysis, as discussed in the preceding paper (Kelly *et al.*, 1979). These circumstances, as well as the constancy of the total carbohydrate concentration during development, render the starving *Dictyostelium* system relatively simple to analyse. Consistent with many previous investigations of this system, the present studies were directed towards the analysis and simulation of the metabolism of unperturbed starving cells. Thus carrier-free tracer concentrations of ^{14}C -labelled amino acid were used as substrates, as this is the endogenous precursor source of tricarboxylic acid-cycle intermediates. (Carrier-free acetate was used in one experiment, but was metabolized so rapidly that cycle intermediates were not labelled.)

In the preceding paper (Kelly *et al.*, 1979) we presented data obtained in this laboratory on metabolite concentrations, ^{14}C -labelled amino acid labelling patterns and O_2 -uptake rates during

differentiation and aging in *Dictyostelium*. In the present paper we examine a model that incorporates some of these data and reveals some aspects of cycle function.

There are various approaches to the analysis of metabolite flux that have been used in the construction of previous models of the tricarboxylic acid cycle. In this study our approach is similar to that of Sauer *et al.* (1970) and Heath & Threlfall (1968). We constructed a network based on established metabolic reactions. Values for some parameters were derived from experimental data (i.e. total pool concentrations, flux through the cycle based on O_2 uptake, and the kinetics of labelling of tricarboxylic acid-cycle intermediates). Others (metabolite compartmentalization and rate constants) were initially given arbitrary values. Parameters that were not determined experimentally were adjusted until a match with the experimental labelling patterns was obtained by using the computer program TFLUX. The program is described in detail elsewhere (Sherwood *et al.*, 1979). Briefly, if we assume that the system is in a steady state, then a set of linear differential equations can be used to describe the specific radioactivities of the metabolite pools over time. From the input the program constructs a system of differential equations of the form $\dot{x} = Ax + b$ in which A is an $n \times n$ constant matrix, b is a constant n vector,

and the n vector χ contains the specific radioactivity of each of n pools. The program then computes each pool specific radioactivity as a function of time. A tape of this program and a user's manual is available upon request.

The Model

The initial step in formulating a model of the tricarboxylic acid cycle was to obtain a value for the flux of material through the cycle. There are several indirect methods of obtaining such a value.

(1) O_2 uptake. The Q_{O_2} at preculmination was $0.16 \mu\text{l}$ of O_2/min per mg dry wt. (Kelly *et al.*, 1979). This is equivalent to an uptake of $7.09 \mu\text{mol}/\text{min}$ per g dry wt. or $0.81 \text{ mm}/\text{min}$, assuming that $1 \text{ g dry wt.} = 8.7 \text{ ml}$ packed-cell volume. If all the O_2 consumed is used in cytochrome oxidation and represents the oxidation of NADH and $FADH_2$ produced as a result of cycle activity, then the flux through the four cycle reactions that result in the reduction of O_2 would be, on average, $0.4 \text{ mm}/\text{min}$. This is of course only an approximation. Some NADH will be generated in the oxidation of amino acids before their entry into the cycle, whereas catabolism of other amino acids involves NADH-consuming reactions.

(2) Protein degradation. If we assume that all the protein catabolized is oxidized through the cycle, we can calculate a value for the cycle flux. By taking White & Sussman's (1961) data of a net utilization of 0.06 mg of protein/h per 10^8 cells and using a value of 132 for the average molecular weight of an amino acid and the value of 0.04 ml packed-cell volume for 10^8 cells, a cycle flux of $0.18 \text{ mm}/\text{min}$ can be obtained.

(3) NH_3 production. Sussman *et al.* (1977) have reported a value of $16 \mu\text{mol}$ of NH_3 produced/ 10^8 cells during the course of differentiation. If we assume that $1 \mu\text{mol}$ of NH_3 represents $1 \mu\text{mol}$ of amino acid deaminated, that the resultant carbon skeleton is completely oxidized, and that 10^8 cells represents 0.04 ml packed-cell volume, then the cycle flux would average about $0.3 \text{ mm}/\text{min}$.

We have used the cycle-flux value calculated from our own O_2 -uptake data, since we feel it is the most accurate indication of cycle activity. Once the rate of cycle flux has been determined the rates of amino acid input to the various tricarboxylic acid-cycle metabolite pools can be determined as follows. We took the amino acid compositions of a number of proteins (Tristram & Smith, 1963), averaged them and used this as an indication of the relative amounts of amino acids that would result from protein degradation in *Dictyostelium*. We then used the known catabolic routes for these amino acids to calculate the amounts of cycle intermediates being produced. Experiments reported by Kelleher *et al.* (1979) have demonstrated that the relative rates of input calculated by this

method are essentially correct. By enzymically degrading the $[^{14}\text{C}]$ citrate produced by $[^{14}\text{C}]$ glutamate or $[^{14}\text{C}]$ aspartate labelling, the ratio (c.p.m. in C-1+C-2)/(c.p.m. in C-3+C-4+C-5+C-6) could be determined over the course of a 30 min labelling. This ratio was constant at about 15% for the $[^{14}\text{C}]$ glutamate experiment, which is in excellent agreement with the ratio calculated from the rate of the 'malic' enzyme reaction and the rates of cyclic input calculated by us.

Once a cycle-flux rate has been set it is possible, with a knowledge of the total cellular metabolite concentrations, to determine their compartmentalization as follows. We assume that (1) our general interpretation of the labelling data is correct (Kelly *et al.*, 1979) and (2) that the pool of 2-oxoglutarate responsible for labelling the cycle has the same specific radioactivity as that isolated. In order to reproduce the initial rapid rise in specific radioactivity observed for all the intermediates, the cycle pool sizes must be small enough to be labelled within 30 s at a flux through the pools of about $0.4 \text{ mm}/\text{min}$. Thus we obtained a maximum value for the aggregate cycle pool size. The size of the metabolite pools outside the cycle is obtained by subtraction. The rate of exchange between cycle and non-cycle pools is assumed to be reflected by the second lower rate of labelling (see Fig. 3 of Kelly *et al.*, 1979). Thus succinate, constant in specific radioactivity after 30 s, is assumed not to exchange with any cytoplasmic pool. 2-Oxoglutarate, also constant in specific radioactivity, either did not exchange or, more likely, the pools of 2-oxoglutarate were labelled to approximately the same extent. This is not unlikely, since (1) the glutamate pool is several orders of magnitude larger than the 2-oxoglutarate pool, and a high exchange rate between these pools has been demonstrated (Randle *et al.*, 1970; Balazs & Haslam, 1965), and (2) glutamate transaminase is normally present in cytoplasmic and mitochondrial compartments at high activity (Lehninger, 1970). Glutamate dehydrogenase has also been shown to exist in *Dictyostelium* in two forms, one in each compartment (Langridge *et al.*, 1977).

Thus we can obtain an approximate calculated value for the cycle flux and, from the $[^{14}\text{C}]$ glutamate-labelling data, an estimate of the compartmentalization of various intermediate pools and their rates of exchange. These data have been used to formulate a model of the cycle shown in Scheme 1. A fuller description of the model will follow a discussion of the $[^{14}\text{C}]$ aspartate-labelling data. In the model shown in Scheme 1 the source of label is a glutamate pool that labels the 2-oxoglutarate pools to constant specific radioactivity ($0.12 \mu\text{Ci}/\mu\text{mol}$). Labelling of 2-oxoglutarate is achieved and maintained by a fast exchange reaction between these pools and the glutamate pool. Labelling of other cycle pools occurs via unidirectional reactions through succinate, fumarate,

malate and citrate. Input to the cycle from protein degradation occurs at 2-oxoglutarate, succinate, fumarate, oxaloacetate and acetyl-CoA. The mode of entry of amino acids into the cycle is based on the complete degradation of a typical protein (Kelleher *et al.*, 1979), and the rate of protein degradation is that determined by White & Sussman (1961). CO₂ is removed at the citrate→2-oxoglutarate, 2-oxoglutarate→succinate and malate→pyruvate reactions. The combined rates of input to the cycle and CO₂ produced are 0.43 mm/min and 1.17 mm/min respectively. These rates are equivalent if expressed in terms of mg of carbon.

[¹⁴C]Aspartate Data

Initial attempts to fit the aspartate data to the model shown in Scheme 1 were unsuccessful. However, inclusion of the following elements, resulting in the configuration shown in Scheme 2, enabled both data sets to be simulated.

(1) Malate dehydrogenase (V+13), fumarase (V+10) and succinate dehydrogenase (V+8) were made reversible reactions. The labelling kinetics of these intermediates suggests that labelling occurs by a reversal of the cycle reactions in which they are involved (Fig. 4 of Kelly *et al.*, 1979). Setting the reverse reaction for malate dehydrogenase (V-13) at 15 times the net forward reaction and the reverse reactions for fumarase (V-10) and succinate dehydrogenase (V-8) at 2-3 times the net forward reaction allowed simulation of the data. These three reactions are known to be reversible (Lowenstein, 1967), and their reversibility has been invoked in other studies (LaNoue *et al.*, 1970; Heath & Threlfall, 1968).

(2) Citrate, and the intermediates labelled from oxaloacetate by reverse reactions (malate, fumarate and succinate), exhibit different labelling kinetics; it was therefore necessary to include in the model two pools of aspartate which give rise to two pools of oxaloacetate of differing specific radioactivities. The first pool (A1) maintains a constant specific radioactivity while labelling oxaloacetate (O1) and citrate (C1). The second pool (A2) is essentially pulse-labelled, giving rise to labelled oxaloacetate (O2), malate (M1), fumarate (F1) and succinate (S1). Duszynski *et al.* (1978) have found evidence for a micro-compartmentalization of aspartate within rat liver mitochondria. Their results imply a concentration gradient of aspartate rather than separate physical compartments. In addition, we have carried out experiments in which [¹⁴C]citrate, labelled from [U-¹⁴C]glutamate and [U-¹⁴C]aspartate, was isolated and converted to the acetate and oxaloacetate moieties from which it was originally derived by the action of citrate lyase and malate dehydrogenase (Kelleher *et al.*, 1979). These experiments indicated

that the specific radioactivities of the cycle pools of malate and oxaloacetate are comparable for the [U-¹⁴C]glutamate experiment, but differ for the [U-¹⁴C]aspartate experiment, with the oxaloacetate specific radioactivity being highest. Bryce *et al.* (1976) concluded from studies of the kinetics of coupled aspartate aminotransferase and malate dehydrogenase that oxaloacetate compartmentalization was the result of an association between these two enzymes. They were able to show that [¹⁴C]oxaloacetate produced by the aspartate aminotransferase reaction did not mix with external unlabelled oxaloacetate, i.e. the [¹⁴C]malate produced from the malate dehydrogenase reaction had the same specific radioactivity as the initial [¹⁴C]aspartate. In addition, Backman & Johansson (1976) using a counter-current distribution technique have found good evidence for an association between these enzymes.

(3) Simulation of the [U-¹⁴C]aspartate data could be accomplished using the experimentally determined pool sizes for glutamate and aspartate only if interactions with other unknown metabolite pools were included. For the glutamate pool a high rate of flux of unlabelled material through the pool was necessary, and for the aspartate pool a rapid exchange with another unlabelled pool was necessary for simulation (see Table 2; V+21, V-21, V+22 and V-22). A high rate of turnover for the glutamate pool was necessary in order to (a) reduce the specific radioactivity of the 2-oxoglutarate pool (2-Kg1) and (b) prevent passage of the label accumulating in the citrate (C1) pool to the succinate (S1), fumarate (F1) and malate (M1) pools.

By using the formulation shown in Scheme 2 both the [¹⁴C]glutamate and [¹⁴C]aspartate labelling data can be simulated. The reactions shown as broken lines in Scheme 2 (V+2, V-2, V+3 and V-3) are included in simulations of the [¹⁴C]glutamate labelling data only and are removed for the [¹⁴C]aspartate simulations, and the reactions shown by dotted lines (V+21 and V-21, V+22 and V-22) are included only in simulations of the [¹⁴C]aspartate data. Pool sizes and reaction rates used in the model are given in Tables 1 and 2. Fig. 1 shows the fit for glutamate and Fig. 2 that for aspartate. In both Figures the experimental data are represented as data points and the simulation by the solid lines. The combined pool sizes for each species indicated in Table 2 are those determined experimentally (Kelly *et al.*, 1979).

Examination of the Model

(1) ATP production

It can be calculated that the model shown in Scheme 2 would produce about 5 mm-ATP/min, if all of the FADH₂ and NADH produced is oxidized by the cytochrome chain. Rutherford & Wright

Scheme 1. Model used in initial simulations of the [^{14}C]glutamate labelling data

(1971) have calculated a value of 12mm/min as a minimum flux through the ATP pool. These calculations were derived from the labelling kinetics of the adenosine phosphate pools by [^3H]adenine. One explanation for this discrepancy is that the calculation by the latter authors included ATP flux due both to tricarboxylic acid-cycle activity and to an active adenylate kinase present in *Dictyostelium*, whereas the value derived from the model represents only the contribution from the tricarboxylic acid cycle to ATP turnover. An ATP flux of 5mm/min is approx. 5-fold that required by the known metabolic demands of the system, such as protein and RNA turnover and the synthesis of new carbohydrate end products of differentiation (Wright & Gustafson, 1972).

(2) Cycle rate

A value for the flux of material through the cycle was obtained as described previously. The rate of flux of material through the cycle (0.4mm/min) is low relative to that estimated for rat liver (1–2mm/min; Sauer *et al.*, 1970; Heath & Threlfall, 1968). However, the Q_{O_2} for starving *Dictyostelium* cells is also relatively low. Increasing the cycle rate in the model 2-fold led to no change in the labelling pattern produced by [^{14}C]glutamate. Decreasing the cycle rate by one-half resulted in a lower rate of incorporation of [^{14}C]glutamate into cycle metabolite pools over the first several minutes.

Table 1. Computer estimate of pool compartmentation

Pool	Identity	Pool size (mm)
2-Kg1	2-Oxoglutarate 1	0.002
2-Kg2	2-Oxoglutarate 2	0.008
UAM1	Unknown amino acid metabolites	Infinite
G	Glutamate	1.2
S1	Succinate 1	0.16
S2	Succinate 2	0.38
S3	Succinate 3	1.10
F1	Fumarate 1	0.008
F2	Fumarate 2	0.033
M1	Malate 1	0.045
M2	Malate 2	0.23
O1	Oxalacetate 1	0.0005
O2	Oxalacetate 2	0.0005
A1	Aspartate 1	0.2
A2	Aspartate 2	0.2
UAM2	Unknown amino acid metabolites	1.8
C1	Citrate 1	0.005
C2	Citrate 2	0.055
Ac	Acetyl-CoA	0.012
Not shown	Protein	Infinite
Not shown	CO_2	Infinite

(3) Compartmentalization

It is assumed that (a) the 2-oxoglutarate pool labelling the cycle has the same specific radioactivity

Scheme 2. Model capable of simulating both the $[^{14}\text{C}]$ aspartate and $[^{14}\text{C}]$ glutamate data

The pools are identified and their sizes given in Table 1. Values for the reaction rates are given in Table 2. The amino acid precursor and CO_2 pools have been omitted for the sake of clarity. Reactions shown in broken lines (V+2, V-2 and V+3, V-3) are used only in simulations of the $[^{14}\text{C}]$ glutamate data and those in dotted lines (V+21, V-21 and V+22, V-22) are used only for the $[^{14}\text{C}]$ aspartate data.

as that isolated, and (b) the initial rapid rise in specific radioactivity reflects entry of the labelled precursor into the cells and its conversion to cycle intermediates. Under these circumstances, in order to simulate the data, the size of the cycle pools (and, by subtraction, the non-cycle pools) is constrained. Malate and fumarate are compartmentalized between cycle and non-cycle pools about 1:5 and citrate

about 1:10. The situation for succinate is more confusing. Three possible configurations for the cellular succinate pool were considered in attempts to simulate the $[^{14}\text{C}]$ glutamate and $[^{14}\text{C}]$ aspartate labelling data. These are shown in Scheme 3.

Glutamate labelling data. In the first configuration (I) there are two pools of succinate, one of which is involved in cycle activity. The size of this cycle pool

Table 2. *Computer reaction rates*

Reaction no.	Identity	Rate (mm/min)
V+1	Glutamate→2-oxoglutarate 1	5.8
V-1	2-Oxoglutarate 1→glutamate	5.8
V+2*	Glutamate→2-oxoglutarate 2	1.7
V-2*	2-Oxoglutarate 2→glutamate	1.7
V+3*	Glutamate→succinate 2	0.80
V-3*	Succinate 2→glutamate	0.80
V+4	2-Oxoglutarate 1→2-oxoglutarate 2	0
V-4	2-Oxoglutarate 2→2-oxoglutarate 1	0
V+5	2-Oxoglutarate 1→succinate 1	0.48
V+6	Succinate 1→succinate 2	0
V-6	Succinate 2→succinate 1	0
V+7	Succinate 2→succinate 3	0
V-7	Succinate 3→succinate 2	0
V+8	Succinate 1→fumarate 1	1.55
V-8	Fumarate 1→succinate 1	1.04
V+9	Fumarate 1→fumarate 2	0.0003
V-9	Fumarate 2→fumarate 1	0.0003
V+10	Fumarate 1→malate 1	1.25
V-10	Malate 1→fumarate 1	0.73
V+11	Malate 1→malate 2	0.004
V-11	Malate 2→malate 1	0.004
V+12	Malate 1→acetyl-CoA	0.14
V+13	Malate 1→oxaloacetate 2	5.59
V-13	Oxaloacetate 2→malate 1	5.21
V+14	Oxaloacetate 2→aspartate 2	5.2
V-14	Aspartate 2→oxaloacetate 2	5.2
V+15	Oxaloacetate 2→oxaloacetate 1	0.38
V+16	Acetyl-CoA→citrate 1	0.42
V+17	Oxaloacetate 1→aspartate 1	0.20
V-17	Aspartate 1→oxaloacetate 1	0.20
V+18	Oxaloacetate 1→citrate 1	0.42
V+19	Citrate 1→citrate 2	0.0002
V-19	Citrate 2→citrate 1	0.0002
V+20	Citrate 1→2-oxoglutarate	0.42
V+21†	Unknown amino acid metabolites→glutamate	16.6
V-21†	Glutamate→unknown amino acid metabolites	16.6
V+22†	Unknown amino acid metabolites→aspartate 2	5.2
V-22†	Aspartate 2→unknown amino acid metabolites	5.2
Not shown	Protein→succinate 1	0.04
Not shown	Protein→fumarate	0.02
Not shown	Protein→acetyl-CoA	0.28
Not shown	Protein→oxaloacetate 1	0.03
Not shown	Protein→2-oxoglutarate 1	0.06
Not shown	Citrate 1→CO ₂	0.42
Not shown	2-Oxoglutarate 1→CO ₂	0.47
Not shown	Malate 1→CO ₂	0.28

* Included only in simulations of the [¹⁴C]glutamate data.

† Included only in simulations of the [¹⁴C]aspartate data.

(S1) must be small relative to the cycle-flux rate, since isolated succinate reaches constant specific radioactivity within 30s. Since the cycle flux rate is 0.4mm/min, S2 must be 0.13mm or less, and since the total cellular succinate pool concentration is 0.61mm, S2 would then be 1.48mm. However, inclusion of such a large unlabelled pool of succinate in the model results in a dilution of label such that the aggregate specific radioactivity of the succinate

pools is an order of magnitude lower than that observed experimentally. Therefore this configuration is excluded.

In the second configuration (II) three pools of succinate are introduced: S1, a small cycle pool; S2, a pool that is labelled independently of S1; S3, a large unlabelled pool. If the pool sizes shown in Scheme 3 (II) are used, then the labelling kinetics for succinate can be reproduced. The additional labelled

Fig. 1. Simulation of the $[^{14}\text{C}]$ glutamate data

The solid lines represent computer output superimposed on the experimental data for malate ($\circ$), 2-oxoglutarate ($\square$), succinate ($\triangle$), fumarate ($\bullet$) and citrate ($\blacktriangle$).

Fig. 2. Simulation of the $[^{14}\text{C}]$ aspartate data

The solid lines represent computer output superimposed on the experimental data for malate ($\circ$), succinate ($\triangle$), fumarate ($\bullet$) and citrate ($\blacktriangle$).

succinate pool (S2) is introduced in order to achieve the experimentally observed specific radioactivity of succinate. In the model this pool is set at 0.38 mM and is labelled to approximately the same extent as the cycle succinate pool. Actually a range of relative sizes of S2 and S3 will reproduce the data if the specific radioactivity of S2 is also varied. The fact that succinate reaches a constant specific radioactivity within 30 s argues for a small S2 pool. In the third configuration (III) the 2-oxoglutarate dehydrogenase reaction is made reversible and a larger pool of succinate (0.5 mM) can be accommodated within the

cycle, since labelling proceeds at a higher rate. This configuration also satisfies the glutamate labelling data.

Aspartate-labelling data. In simulating the $[^{14}\text{C}]$ -aspartate data all three possible configurations were examined. If the size of S1 is increased slightly the first configuration will reproduce the data. The second configuration will also result in simulation of the data if S2 (labelled by $[^{14}\text{C}]$ glutamate) is not labelled by $[^{14}\text{C}]$ aspartate. The third configuration will not enable simulation, since, if the 2-oxoglutarate dehydrogenase reaction is made reversible, label loss from the succinate pool during $[^{14}\text{C}]$ aspartate labelling does not occur to the same extent as observed experimentally.

We conclude that succinate exists in configuration (II) with three compartments: S1, the cycle pool; S2, a pool labelled by $[^{14}\text{C}]$ glutamate but not by $[^{14}\text{C}]$ -aspartate; S3, a large pool not labelled by either.

The fast exchange rate glutamate $\rightleftharpoons$ 2-oxoglutarate (2-Kg1) makes an assessment of 2-oxoglutarate compartmentalization impossible.

Attempts have been made to estimate compartmentalization directly by measuring the concentrations of tricarboxylic acid-cycle intermediates in cellular fractions (Tischler *et al.*, 1977). Assuming a cytosolic to mitochondrial volume ratio of about 10:1, a comparison of our compartmentalization ratios with theirs shows agreement for the dicarboxylic acid malate. However, they conclude that citrate is largely located in the mitochondrion, whereas our studies indicate that citrate is largely cytosolic. This discrepancy may be explained by the fact that our method distinguishes only between cycle-active and cycle-inactive material. Hence our 'compartmentalization' is between cycle metabolite pools and non-cycle pools. Our results would be compatible with those of Tischler *et al.* (1977) if only a small proportion of mitochondrial citrate is involved in cycling. This would be the case if, for example, an association between citrate synthase and aconitase existed which resulted in a transfer of metabolite from one enzyme to the other (Srere & Mosbach, 1974). Several previous attempts to model tricarboxylic acid-cycle activity have also reported anomalous labelling behaviour for citrate (Stucki & Walter, 1972; Müllhoffer *et al.*, 1977). The latter investigators raise the possibility that two mitochondrial pools of citrate may exist which are labelled by different substrates.

(4) Exchange reactions

The succinate pools do not appear to exchange material, whereas the fumarate and citrate pools do, at approximately the same low rate (0.0003 mM/min). Malate pools show the highest rate of exchange, at 0.004 mM/min. A high rate for the mitochondrial-

Scheme 3. Possible configurations for the cellular succinate pool
See the text for details.

cytosolic exchange of malate has been demonstrated in other systems (Rognstad & Katz, 1973) and may be related to the functioning of the malate/aspartate shuttle, which is involved in the transfer of reducing equivalents from the cytosol to the mitochondrion (Krebs *et al.*, 1967). The above exchange reactions may actually be unidirectional transfer of labelled material from the cycle pool to cytosolic pools. The reverse reaction is included here to maintain a steady state. The exchange reaction for fumarate may be a labelling of a fumarate pool by malate that has escaped from the cycle via a reversal ($V-10$) of the fumarase reaction ($V+10$).

(5) 'Malic' enzyme

The inclusion of the 'malic' enzyme reaction ($V+12$) was necessitated by the nature of the material entering the cycle when protein is the major energy source. The amino acids resulting from protein degradation give rise to a number of cycle metabolites, which are predominantly 4- or 5-carbon intermediates. A proportion of these must be converted to acetyl-CoA. The existence of such a reaction is supported by the following: (a) if citrate isolated from [^{14}C]glutamate-pulsed cells is degraded by using citrate lyase and the resulting acetate and malate are isolated, then 15% of the total radioactivity

in citrate is found in the acetate fraction derived from acetyl-CoA; (b) 'malic' enzyme, which catalyses the conversion of malate to pyruvate, has been isolated from *D. discoideum*. Its isolation and properties are discussed in detail elsewhere (Kelleher *et al.*, 1979). Removal of this reaction from the model results in only a 25% decrease in the specific radioactivity of citrate and negligible decreases in the specific radioactivities of other cycle metabolites.

(6) Unknown amino acid metabolism

In simulating the [^{14}C]glutamate data the glutamate pool was held at a constant specific radioactivity and pool turnover was not critical. However, simulation of the [^{14}C]aspartate data necessitated a large flux (16mm/min) of unlabelled material through the glutamate pool. Simulation of the [^{14}C]aspartate data also necessitated a rapid exchange between aspartate (A2) and an unidentified unlabelled pool (UAM2). Although these reactions are shown as interactions with other amino acid pools, their biochemical basis *in vivo* is unknown. The [^{14}C]glutamate data cannot be simulated with the high rate of glutamate turnover used in the [^{14}C]aspartate simulations. The compartmentalization of the glutamate pool and the relationship of these compartments to the high rate of protein turnover in *Dictyostelium* may be responsible for these effects.

The model of tricarboxylic acid-cycle activity in *Dictyostelium* described in this paper is preliminary in nature. It has made many specific predictions which can serve as the basis for further studies on energy metabolism, particularly enzyme and metabolite compartmentalization.

This work was supported by U.S. Public Health Service grants no. AG00433 and AG00260 from the National Institutes of Health and by a U.S. Public Health Service post-doctoral fellowship no. AG05065 to J. K. K.

References

- Backman, L. & Johansson, G. (1976) *FEBS Lett.* **65**, 39–43
 Balázs, R. & Haslam, R. J. (1965) *Biochem. J.* **94**, 131–141

- Bryce, C. F. A., Williams, D. C., John, R. A. & Fasella, P. (1976) *Biochem. J.* **153**, 571–577
 Duszynski, J., Mueller, G. & LaNoue, K. (1978) *J. Biol. Chem.* **253**, 6149–6157
 Heath, D. F. & Threlfall, C. J. (1968) *Biochem. J.* **110**, 337–362
 Kelleher, J. K., Kelly, P. J. & Wright, B. E. (1979) *J. Bacteriol.* **138**, 467–474
 Kelly, P. J., Kelleher, J. K. & Wright, B. E. (1979) *Biochem. J.* **184**, 581–588
 Krebs, H. A., Gascoyne, J. & Hotton, B. M. (1967) *Biochem. J.* **102**, 275–282
 Langridge, W. H. R., Komuniecki, P. & DeToma, F. J. (1977) *Arch. Biochem. Biophys.* **178**, 581–587
 LaNoue, K., Nicklas, W. J. & Williamson, J. R. (1970) *J. Biol. Chem.* **245**, 102–111
 Lehninger, A. L. (1970) *Biochemistry*, 1st edn., Worth, New York
 Lowenstein, J. M. (1967) *Metab. Pathways 3rd Ed.* **1**, 147–254
 Müllhofer, G., Müller, C., Von Stetten, C. & Gruber, E. (1977) *Eur. J. Biochem.* **75**, 331–341
 Randle, P. J., England, P. J. & Denton, R. M. (1970) *Biochem. J.* **117**, 677–695
 Rognstad, R. & Katz, J. (1973) *Biochem. J.* **132**, 349–352
 Rutherford, C. L. & Wright, B. E. (1971) *J. Bacteriol.* **108**, 269–275
 Sauer, F., Erfle, J. D. & Binns, M. R. (1970) *Eur. J. Biochem.* **17**, 350–353
 Sherwood, P., Kelly, P. J. & Wright, B. E. (1979) *Comp. Biomed. Res.* **10**, 66–74
 Srere, P. A. & Mosbach, K. (1974) *Annu. Rev. Microbiol.* **28**, 61–83
 Stucki, J. W. & Walter, P. (1972) *Eur. J. Biochem.* **30**, 60–72
 Sussman, M., Schlinder, J. & Kim H. (1977) in *Development and Differentiation in the Cellular Slime Moulds* (Cappuccinelli, P. & Ashworth, J. M., eds.), pp. 31–51, Elsevier/North-Holland, Amsterdam and New York
 Tischler, M. E., Hecht, P. & Williamson, J. R. (1977) *Arch. Biochem. Biophys.* **181**, 278–293
 Tristram, G. R. & Smith, R. H. (1963) *The Proteins* **1**, 45–57
 White, G. J. & Sussman, M. (1961) *Biochim. Biophys. Acta* **53**, 285–293
 Wright, B. E. & Gustafson, G. L. (1972) *J. Biol. Chem.* **247**, 7875–7884