Metabolic Control Mechanisms

VII. A DETAILED COMPUTER MODEL OF THE GLYCOLYTIC PATHWAY IN ASCITES CELLS*

DAVID GARFINKEL† AND BENNO HESS

From the Johnson Research Foundation, University of Pennsylvania, Philadelphia 4, Pennsylvania, and the Chemical Laboratory, University Medical Clinic, University of Heidelberg, Heidelberg, Germany

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A computer model of the glycolytic and respiratory pathway of Ehrlich ascites cells has been described in a previous paper of this series (4). This model qualitatively represented aerobic glycolysis, primarily in the direction from glucose to lactate. It was found that such specific features of the metabolic regulation of these cells as the Pasteur and Crabtree effects could be accounted for by assuming that adenosine triphosphate is compartmented between the cytoplasm and the mitochondria, and that the adenine nucleotides are among the most important regulatory substances. This model was as complete as was permitted by the limited memory capacity of the computer used and by the limitations of the available experimental data for Ehrlich ascites cells.

Since the construction of this model, much more information on metabolic patterns of these cells has become available (5, 6). The authors have also been able to obtain access to a somewhat more powerful computer. It has accordingly been possible to correct some of the deficiencies of the previous model, such as omission or lumping together of some enzymes and intermediates of the pathway, omission of reverse reactions and detailed enzyme mechanisms, and selection of the amounts of some enzymes largely for convenience of calculation.

This communication presents an attempt to construct a reasonably complete and detailed model which is in quantitative agreement with the available data, although this is probably not a unique representation of the data. There is no single preparation of ascites cells for which all the necessary data have been obtained, and construction of this model was started from one preparation for which the values of all the glycolysis substrate, product, and intermediate concentrations were available, supplemented by reference to the large body of data presently available on the behavior of glycolysis in ascites cells, both in other previous papers of this series (7-11) and in other sources in the literature (12-16).

EXPERIMENTAL PROCEDURE

Method

The basic computer methods used in preparing this model have been previously described (4, 17, 18). Calculations were at first performed with the Univac I computer; as the memory capacity of this computer proved to be insufficient for this study, part of the work was performed with the Univac II computer, which has twice the memory capacity. As the authors’ access to a Univac II is limited (one visit per week), much of the work here described was carried out with the Univac I computer, by dividing the model into portions of three or four enzymes each and bringing each portion to a state of satisfactory behavior. These portions were then merged to form a complete model which was studied with the Univac II.

The initial input to the computer consists of the chemical equations constituting the model (listed in Table I), in which the chemical names are represented by three-letter abbreviations (tabulated in Table II). Each equation is treated as unidirectional, and the reverse reaction must be written separately. The disappearance of a chemical in a reaction is indicated by listing it with a minus sign on the right side of the equation (which may be independent of the left side). From these chemical equations the computer will formulate the appropriate differential equations according to the law of mass action.

These differential equations are solved by a modified Euler point-slope method after rate constants, concentrations, etc., are specified. The size of the integration step used in solving the equations is so regulated as to keep the error of the solution constant throughout (throughout the work here described this error has been taken as 1%, which is also the greatest accuracy to which the data can be reported in graphical form). Since the Univac I and II computers cannot easily represent quantities greater than 1, all concentrations are “normalized” by dividing them by a maximal allowable limit. In Table I both the initial value and the maximal limit for each chemical are shown the first time it is mentioned.

One source of serious difficulty which was encountered (to a much smaller extent) in past work is a tendency for the variables in the differential equations which represent substances with high turnover numbers (usually enzymes or enzyme-substrate intermediates) to break into oscillations during the solution of the differential equations. This is illustrated in Fig. 1. Detailed mathematical analysis indicates that such oscillations are likely to arise in differential equations of this type when enzyme turnover numbers from the literature are inserted, and that this does not depend on the method of solving the equations. Thus far no really satisfactory remedy has been found. In view of the extent to which these oscillations slow down the course of calculation,1 a number of expedients were adopted to speed

1 The first time the complete glycolysis system was tried on the
TABLE I
Glycolysis system equations

Equations are grouped logically. The numbers of the equations represent the order in which they were given the computer. These are not consecutive because new equations are normally added at the end of the array rather than being interpolated. The calculated results are independent of the order of the equations. The significance of the items in boldface type is explained in the text. The abbreviations are defined in Table II.

Hexokinase

$$0 \rightarrow 1 \times 10^{-2} \text{GLU} + 3.04 \times 10^{-3} \text{HKG} + 3.7 \times 10^4$$
$$1 \times 10^{-2} \text{HKG} \rightarrow \text{GLU} - \text{HKS}$$

$$1.52 \times 10^{-7} \text{HKG} + 1.5 \times 10^9 \rightarrow \text{GLU} + \text{HKS} - \text{HKG}$$

$$1.52 \times 10^{-7} \text{HKG} + 5 \times 10^{-3} \text{1TP} \rightarrow \text{HKG} - \text{HKS} - \text{1TP}$$

$$5.72 \times 10^{-7} \text{HKG} + 6.5 \times 10^9 \rightarrow \text{HKG} + \text{1TP} - \text{HKS}$$

$$2 \times 10^{-9} \text{HKG} \rightarrow \text{HKS} + \text{GLP} - \text{HKS}$$

$$1 \times 10^{-10} \text{AdP} + \text{HKG} = 2 \times 10^{-9} \text{HKP} - \text{AdP} - \text{HKG}$$

$$1 \times 10^{-10} \text{AdP} + \text{HKG} = 2 \times 10^{-9} \text{HKP} - \text{AdP} - \text{HKG}$$

$$1 \times 10^{-10} \text{AdP} + \text{HKG} = 2 \times 10^{-9} \text{HKP} - \text{AdP} - \text{HKG}$$

$$1 \times 10^{-10} \text{AdP} + \text{HKG} = 2 \times 10^{-9} \text{HKP} - \text{AdP} - \text{HKG}$$

Phosphohexose isomerase

$$\text{GLP} + 1.5 \times 10^{-8} \text{ISM} \rightarrow 8.7 \times 10^6 \text{IGS} - \text{GLP} - \text{ISM}$$

$$8.5 \times 10^{-8} \text{IGS} + 1.57 \times 10^9 \rightarrow \text{CLP} + \text{ISM} - \text{IGS}$$

$$1.01 \times 10^{-9} \text{CLP} + 1.01 \times 10^{-7} \rightarrow \text{FRP} + \text{ISM} - \text{CLP}$$

$$1.64 \times 10^8 \text{FRP} + 3.2 \times 10^6 \rightarrow \text{ISG} - \text{FRP} - \text{ISM}$$

$$2 \times 10^{-14} \text{FRP} + 5 \times 10^{-8} \rightarrow \text{FRP} + 3.2 \times 10^6 \rightarrow \text{ISG} - \text{FRP} - \text{ISM}$$

Phosphofructokinase

$$\text{FRP} + 6.58 \times 10^{-4} \text{PPK} \rightarrow 1.91 \times 10^{11} \text{PFF} - \text{PPK} - \text{FRP}$$

$$\text{FRP} + 6.58 \times 10^{-4} \text{PPK} + 1.91 \times 10^{11} \rightarrow \text{PFF} - \text{PPK} - \text{FRP}$$

$$\text{FRP} + 6.58 \times 10^{-4} \text{PPK} + 1.91 \times 10^{11} \rightarrow \text{PFF} - \text{PPK} - \text{FRP}$$

Univac II, + hour of computer time was needed to simulate 75 milliseconds of real time.

$$2.8 \times 10^{-8} \text{PFF} + 1.4 \times 10^3 \rightarrow \text{PPK} + \text{FRP} - \text{PFF}$$

$$\text{PFF} + 1.1 \times 10^4 \rightarrow \text{ADP} + \text{FPP} + \text{PPK} - \text{1TP} - \text{PFI}$$

$$2.8 \times 10^{-8} \text{PFF} + 1.5 \times 10^{-4} \text{FPP} \rightarrow 9 \times 10^6 \text{PFI} - \text{PPF}$$

$$0 \rightarrow 7 \times 10^{-8} \text{PFI} \rightarrow 8 \times 10^4 \rightarrow \text{PFF} + \text{FPP} - \text{PFI}$$

$$\text{PFI} + \text{ADP} \rightarrow 9 \times 10^6 \rightarrow \text{PFF} + \text{PFI} - \text{FPP}$$

Aldolase

$$\text{FPP} + 1.0 \times 10^{-4} \rightarrow \text{ALD} 3.11 \times 10^9 \rightarrow \text{ALF} - \text{FPP}$$

$$5.25 \times 10^{-8} \text{ALF} \rightarrow 4.02 \times 10^9 \rightarrow \text{ALD} + \text{FPP} - \text{ALF}$$

$$\text{ALF} 5.24 \times 10^9 \rightarrow \text{ALA} + \text{GAP} - \text{ALF}$$

$$1.7 \times 10^{-8} \text{GAP} + 3.3 \times 10^9 \rightarrow \text{ALF} - \text{GAP}$$

$$1 \times 10^{-8} \text{DHA} + 8.33 \times 10^6 \rightarrow \text{ALA} - \text{DHA}$$

$$1 \times 10^{-8} \text{DHA} + 8.33 \times 10^6 \rightarrow \text{ALA} - \text{DHA}$$

Triosephosphate isomerase

$$\text{DHA} + 4 \times 10^{-8} \text{TBM} \rightarrow 1.29 \times 10^4 \rightarrow \text{TID} - \text{DHA}$$

$$5.44 \times 10^{-4} \text{TID} \rightarrow 1.14 \times 10^6 \rightarrow \text{GAP} + \text{TIM} - \text{TID}$$

$$\text{TID} 2.5 \times 10^2 \rightarrow \text{TID} + \text{DHA} - \text{TID}$$

$$\text{GAP} + \text{TIM} 2.01 \times 10^4 \rightarrow \text{TID} - \text{GAP} - \text{TID}$$

$$\text{ALD} + \text{TBD} - \text{ALA} - \text{TBM}$$

$$\text{TBD} + \text{ALD} 1.5 \times 10^9 \rightarrow \text{ALA} + \text{TBM} - \text{TBD}$$

$$5 \times 10^{-8} \text{TBD} - 1.36 \times 10^9 \rightarrow \text{TBM} + \text{DHA} - \text{TBD}$$

$$\text{TBD} 6.6 \times 10^9 \rightarrow \text{TBM} + \text{GAP} - \text{TBD}$$
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<thead>
<tr>
<th>Reaction</th>
<th>Rate Constant</th>
<th>Product(s)</th>
<th>Reaction</th>
<th>Rate Constant</th>
<th>Product(s)</th>
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<tr>
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<tr>
<td>TIM + DHA → TBD - TIM</td>
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<td>$2 \times 10^{-6}$ MOB + $3 \times 10^{-6}$ PIΔ $5.9 \times 10^6$</td>
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<tr>
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<td></td>
<td>MOX + DGA - MOB - PIΔ</td>
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<tr>
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<td>MOD → $7.85 \times 10^9$</td>
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<td>$3.35 \times 10^6$ DGA + PGK - PGG</td>
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<td>PGK + $3 \times 10^{-9}$ 1TP + $3 \times 10^{-9}$ PGK - PGG - ADP</td>
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<td>PGK + ADP $1.33 \times 10^7$</td>
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<td></td>
<td>$1 \times 10^{-10}$ 1TP + $3 \times 10^{-9}$ PGK - PGG - ADP</td>
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<td>$1 \times 10^{-9}$ 3GA + $1 \times 10^{-4}$ 1TP + PGK $2.2 \times 10^8$</td>
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<td>$1 \times 10^{-9}$ 3GA + $1 \times 10^{-4}$ 1TP + PGK $2.2 \times 10^8$</td>
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<td>Phosphoglyceromutase</td>
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<td>3GA + DGA - MOB - PIΔ</td>
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<td>4 × 10^{-5}</td>
<td>4 × 10^{-5} 1TP + LDD $4.72 \times 10^6$</td>
<td>LDL - PYR</td>
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<tr>
<td>4 × 10^{-4}</td>
<td>4 × 10^{-4} 1TP + LDD $4.72 \times 10^6$</td>
<td>LDL - PYR</td>
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<td>Lactate dehydrogenase</td>
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<tr>
<td>LDN + $6.66 \times 10^{-3}$ LAC $1.5 \times 10^4$</td>
<td>LDL - LDN</td>
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<td>Lactate dehydrogenase</td>
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<td>LDN + $6.66 \times 10^{-3}$ LAC $1.5 \times 10^4$</td>
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<td>Lactate dehydrogenase</td>
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</tbody>
</table>
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LDH + DPN $\xrightarrow{4.5 \times 10^5}$ LDN - LDH - DPN (66)

$5 \times 10^{-8}$ LDN $\xrightarrow{1.88 \times 10^4}$ LDH + DPN - LDN (67)

$1.5 \times 10^{-4}$ LDH + DPH $\xrightarrow{1.11 \times 10^6}$ LDD - LDH - DPH (68)

$3 \times 10^{-8}$ LDD $\xrightarrow{6.5 \times 10^9}$ LDH + DPH - LDD (69)

Mitochondrial oxidative phosphorylation

PYR + $2 \times 10^{-4}$ DIN $\xrightarrow{7 \times 10^4}$ DIH - DIN - PYR (70)

$4 \times 10^{-3}$ DIH + $2 \times 10^{-4}$ XI + $5 \times 10^{-4}$ OXY $\xrightarrow{2.5 \times 10^8}$ DIN + XSI - OXY - XI - DIH (71)

$2 \times 10^{-5}$ XSI + PIA $\xrightarrow{6.8 \times 10^3}$ XSP - XSI - PIA (72)

$2 \times 10^{-3}$ XSP + ADP + ADP $\xrightarrow{3.3 \times 10^9}$ 2TP + XI - XSP - ADP (73)

XSI + 0.25 $\times 10^{-7}$ DBP $\xrightarrow{4 \times 10^9}$ XI - XSI (74)

$5 \times 10^{-1}$ 2TP + DBP $\xrightarrow{1 \times 10^1}$ 1TP - 2TP (75)

X.I + 2TP $\xrightarrow{6 \times 10^8}$ XSI + ADP + PIA - XI - 2TP (76)

Hydrolysis of mitochondrial ATP

CON $\xrightarrow{2}$ ADP + PIA + 2TP (79)

Cytoplasmic ATP utilization

1TP + $2 \times 10^{-6}$ PUE $\xrightarrow{6 \times 10^4}$ PPP - 1TP (76)

$1 \times 10^{-3}$ PPP $\xrightarrow{3.06 \times 10^4}$ PUE + ADP + PIA (77)

$\alpha$-Glycerophosphate dehydrogenase

DHA + DPH $\xrightarrow{1.67 \times 10^6}$ ACP + DPN - DHA (79)

$1.09 \times 10^{-1}$ 2TP + AGP + DPN $\xrightarrow{6 \times 10^8}$ DHA + DPH - AGP - DPN

Glucose 6-phosphate synthesis

$1 \times 10^{-3}$ CON $\xrightarrow{3 \times 10^{-3}}$ GLP (80)

the calculation. Errors introduced by these expedients are
described under the appropriate expedient. The oscillations
themselves usually affect only the speed of the computation,
not the results.

1. Reduction of the turnover number of the enzyme (by
decreasing all the rate constants and proportionally increasing
the amount of enzyme). Table III shows the extent to which
this has been done for those enzymes whose turnover number
is available from the literature. In nearly all cases at least one
check was made to determine whether changing the turnover
number had any effect on the behavior of the model, and only
in the case of glyceraldehyde 3-phosphate dehydrogenase (where
the amount of one of the substrates, DPNH, is comparable to
the amount of the enzyme itself) was there any effect. The
turnover number for this enzyme was therefore kept at nearly
the literature value. The turnover numbers used in the preced-
ing model (4) are generally smaller than those in the present
model.

2. It was in effect possible to increase the ratio of the amount
of enzymatic work done by each enzyme to the amount of oscillat-
ing it did, by making 100 molecules of substrate disappear in
a reaction with 1 molecule of enzyme and 100 molecules of
product appear, instead of 1 molecule of substrate and 1 molecule
of product. Corrections to accomplish this were included in
the initial conditions. The substances so multiplied by 100 are
indicated in boldface type in Table I. Since the memory
capacity of the Univac II is insufficient to permit the inclusion
of all the required correction factors, two of them were omitted
(for the consumption of ADP in the pyruvate kinase reaction
(Reactions 59 and 60)), resulting in an apparent slow synthesis
of adenine nucleotides. Results shown in Fig. 2 are corrected
for this apparent synthesis (by hand) during the second half of
the solution.

3. When the constant error criterion mentioned above per-
mitted a long integration step, the length of this step was in-
creased by a factor of 10.

4. An enzyme displaced from its proper value during an
oscillation will return to it in a number of short steps. Such an
enzyme can be approximately returned to its proper value with
one integration step of a characteristic length (independent of
the amplitude of the oscillation, but slowly changing with time).
At first such characteristic step lengths, calculated manually,
were inserted into the computer as lower limits for the integration
step size. Later a program was written which calculates four
such minimal step sizes and imposes them (in order of increasing
size) as needed.

In one instance (Fig. 3), the amount of phosphohexose isomer-
ase was altered somewhat (this is automatically detected by the
computer) when this enzyme started to oscillate.

5. Since the identity of the oscillating enzymes changes with
time, the computation was stopped whenever the rate of progress
decreased, and the program mentioned immediately above was
reset to apply to the enzymes that were found to be oscillating
at that time. Calculation was resumed later from that point.
The computation was thus carried along from time to time until
a sufficiently long time interval had been covered to permit useful
TABLE II
Listing of abbreviations, in order mentioned in Table 1*

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Enzyme</th>
<th>Abbreviation</th>
<th>Enzyme</th>
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<tbody>
<tr>
<td>GLU</td>
<td>Glucose</td>
<td>3GA</td>
<td>3-Phosphoglycerate</td>
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<tr>
<td>HKS</td>
<td>Free hexokinase</td>
<td>PGA</td>
<td>Phosphoglyceromutase activated by diphosphoglyceric acid</td>
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<td>Hexokinase-glucose complex</td>
<td>PGP</td>
<td>Phosphoglyceromutase-phosphoglyceric acid complex</td>
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<td>1TP</td>
<td>Cytoplasmic ATP</td>
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<td>2-Phosphoglycerate</td>
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<td>Apophosphoglyceromutase</td>
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<td>Hexokinase-glucose-6-phosphate complex</td>
<td>ENL</td>
<td>Free enolase</td>
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<td>Free phosphohexose isomerase</td>
<td>ENP</td>
<td>Enolase-phosphoenolpyruvate complex</td>
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<td>Hexokinase-ADP (inhibited) complex</td>
<td>PEP</td>
<td>Phosphoenolpyruvate</td>
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<td>Phosphohexose isomerase-hexose phosphate complex</td>
<td>ENI</td>
<td>Complex of enolase and 3-phosphoglycerate (inhibited)</td>
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<td>PYK</td>
<td>Free pyruvate kinase</td>
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<td>PYD</td>
<td>Pyruvate kinase-ADP complex</td>
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<td>GAP</td>
<td>Glyceraldehyde phosphate</td>
<td>LDH</td>
<td>Free lactic dehydrogenase</td>
</tr>
<tr>
<td>DHA</td>
<td>Dihydroxyacetone phosphate</td>
<td>DIN</td>
<td>Mitochondrial DPN</td>
</tr>
<tr>
<td>TIM</td>
<td>Free triosephosphate isomerase</td>
<td>DIH</td>
<td>Mitochondrial DPN</td>
</tr>
<tr>
<td>TID</td>
<td>Complex of triose phosphate and TIM</td>
<td>X.I</td>
<td>Low energy intermediate in oxidative phosphorylation</td>
</tr>
<tr>
<td>TBM</td>
<td>Triosephosphate isomerase bound to aldolase</td>
<td>OXY</td>
<td>Oxygen</td>
</tr>
<tr>
<td>TBD</td>
<td>Complex of triose phosphate and TIM</td>
<td>XSI</td>
<td>High energy intermediate in oxidative phosphorylation</td>
</tr>
<tr>
<td>MOD</td>
<td>Glyceraldehyde phosphate dehydrogenase-DPN complex</td>
<td>XSP</td>
<td>Phosphorylated intermediate in oxidative phosphorylation</td>
</tr>
<tr>
<td>MOB</td>
<td>Acyl enzyme intermediate of glyceraldehyde phosphate dehydrogenase</td>
<td>ZTP</td>
<td>Mitochondrial ATP</td>
</tr>
<tr>
<td>DPH</td>
<td>DPNI</td>
<td>DBP</td>
<td>Dibromophenol</td>
</tr>
<tr>
<td>PIA</td>
<td>Inorganic phosphate</td>
<td>PUE</td>
<td>Cytoplasmic ATP-utilizing enzymes</td>
</tr>
<tr>
<td>MOX</td>
<td>Free glyceraldehyde phosphate dehydrogenase</td>
<td>PPP</td>
<td>Phosphorylated form of PUE</td>
</tr>
<tr>
<td>DGA</td>
<td>1,3-Diphosphoglycerate</td>
<td>AGP</td>
<td>α-Glycerophosphate</td>
</tr>
<tr>
<td>PGK</td>
<td>Free phosphoglycerokinase</td>
<td>CON</td>
<td>Fictitious chemical used to make reactions proceed at a constant rate</td>
</tr>
<tr>
<td>PGG</td>
<td>Complex of phosphoglycerokinase and diphosphoglyceric acid</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* ADP and DPN have their usual meanings.

Comparison with experimental results. For the total of 50 seconds of real time represented in Fig. 2, approximately 7 to 8 hours of computer time (distributed over several visits to the computer) were required. It is therefore impractical to repeat a long calculation any number of times.

Construction of Model

The computer model was constructed by addition to the previously published model (4) of detailed kinetic mechanisms (obtained from the literature as described below) and necessary reverse reactions. The experimental situation being simulated is an ascites cell preparation at 22°, which is initially in the endogenous steady state (with a high ratio of ATP to ADP). After 4 seconds, glucose is added and the system is followed as far as practicable, which in these experiments did not extend as far as the glucose-saturated steady state. A simulation of this steady state was therefore carried out separately; after 4 seconds in this steady state, an uncoupling agent (dibromophenol) is added and the resulting transient changes followed.
cells at 20° (7-11); (c) accumulated experience with ascites cells in the authors’ laboratories,2 and (d) two specific preparations of ascites cells described by Hess (5, 19). The model constructed is effectively a simulation of one of these preparations for which the over-all fluxes and concentrations of nearly all the important chemicals are available. Such values of concentration and other information (lactate production, oxygen uptake, α-glyceraldehyde phosphate production) which could not be obtained for this preparation were filled in from the other preparation and from other sources of information. The experiments on the preparation being simulated were started in the endogenous steady state (with adequate oxygen and phosphate) at 22°, then glucose was added and the system was sampled in the glucose-saturated steady state 5 minutes later, when the glucose uptake was measured as 27.2 μmoles per kg of cells, wet weight, per second.

Construction of the model was started by writing the appropriate chemical equations (mostly obtainable from the literature).3 Calculation of the numbers to be associated with these equations began with a consideration of the glucose-inhibited steady state condition. Since this is a steady state, the net flux through those enzymes of the glycolytic pathway that handle hexoses must be equal to the net flux for glucose (27.2 μmoles per kg, wet weight, per second), and the net flux through the triose-handling enzymes must be twice this value. The net flux is the difference between the forward and reverse fluxes for each enzyme. The ratio between the forward and backward fluxes for each enzyme is obtained by comparing the steady state concentrations of substrates and products with the corresponding thermodynamic equilibrium concentrations. At equilibrium the forward and backward fluxes are equal; if the substrates of a given enzyme are present in greater than equilibrium amounts, as compared to the products, the forward flux will be proportionally larger. The calculation of the ratio of fluxes from the ratio of concentrations has been described more thoroughly elsewhere.4

Once the fluxes are known, it is possible to compute rate constants to be associated with the chemical equations. Such Michaelis constants, turnover numbers, and rate constants as are known were obtained from the literature and combined with the fluxes to yield a (nonunique) set of values for the rate constants. When used in the model, these values enabled it to maintain the steady state concentrations which were experimentally observed in the glucose-inhibited steady state (19).

The model described in the preceding paragraph was then altered to maintain the observed endogenous steady state also.

This could not be done unless some additional source of glucose phosphate was postulated. The phosphofructokinase reaction is very far from equilibrium in the endogenous steady state, hexose monophosphates being present in excess. Unless phosphofructokinase is essentially inactive in the endogenous steady state, there must be a net flux through this enzyme. Although the high ATP:ADP ratio does indicate low phosphofructokinase activity (20, 21), the degree of inhibition required to account for the observed endogenous concentrations seems less probable than some synthesis of glucose phosphate from such sources as glycogen and the ribose shunt pathway. This flux has been assumed to be about 10 μmoles per second per kg of cells, wet weight; the numerical value depends on the phosphofructokinase mechanism used. This formation of glucose phosphate (represented by Equation 86 in Table I) is assumed to be suppressed by excess glucose.

Once the model was able to hold both the endogenous and the glucose-saturated steady state, it was then made to follow the appropriate transient kinetics on going from one to the other, after the addition of glucose. The first model did not do this at all satisfactorily, and it was necessary to work down the chain of enzymes, starting with hexokinase, modifying each so that it yielded the proper behavior on glucose addition.

Hexokinase—It has been suggested (12, 15, 22) that hexokinase plays an important regulatory role in glycolysis, being regulated by glucose 6-phosphate inhibition. However, this does not account for the fact that when an uncoupling agent is added to ascites cells in the glucose-saturated state (where the glucose 6-phosphate concentration is high) the uptake of glucose increases strongly, but the concentration of glucose 6-phosphate falls only slightly (19). After a number of unsatisfactory attempts to modify the yeast hexokinase mechanism (23, 24) to include glucose 6-phosphate inhibition, the work of Hammes and Kochavi (25-27) on yeast hexokinase came to our attention, in which it is shown that there is also a product inhibition by ADP.6 Their mechanism was incorporated into the model (Equations 1 through 10 of Table I), and the resulting behavior of glucose 6-phosphate was found to fit the experimental data reasonably well.

The rate constants originally obtained in the process of fitting the experimental data empirically (Table I) were identical with those obtained by Hammes and Kochavi (25-27), except for a constant factor representing a difference in turnover number. In view of computer memory space limitations, the effects of magnesium ion on hexokinase could not be included in this model.

Phosphohexose Isomerase—The next enzyme in the pathway, phosphohexose isomerase, is not generally regarded as limiting; it is very nearly in equilibrium in the glucose-saturated steady state (19), and addition of this enzyme to extracts of ascites cells does not increase the rate of glycolysis (12). The simplest possible mechanism was used in this model (Equations 11 through 14, Table I).

Phosphofructokinase—The literature does not contain any satisfactory mechanism for phosphofructokinase, an enzyme which is most difficult to study experimentally. Straightforward mechanisms for this enzyme, when tried in the model, did not yield enough enzymatic activity during the initial transients after the addition of glucose (when fructose 1,6-diphosphate is being

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1 B. Hess and G. Gey, in preparation.
2 For all enzymes having more than one substrate, compulsory order mechanisms were used, as these can be represented in fewer equations than random order mechanisms. The errors introduced thereby are probably not serious.
3 For all enzymes having more than one substrate, compulsory order mechanisms were used, as these can be represented in fewer equations than random order mechanisms. The errors introduced thereby are probably not serious.
4 The courtesy of Dr. Hammes in allowing us to see these manuscripts before publication is gratefully acknowledged.
5 Dr. P. K. Maitra has recently found that the hexokinase of ascites cells is inhibited about 50% by 1 mM ADP.
formed very rapidly), and yielded too much activity during the endogenous steady state (as compared to the glucose-saturated steady state). The available Michaelis constant from the literature, \( 2.7 \times 10^{-4} \text{ M} \) (28), indicates that this is not due to a shortage of ATP, since the ATP concentration of the cytoplasm probably exceeds this under all glycolysis conditions. High activity is observed in the presence of high cytoplasmic ATP (when glucose is added to the endogenous steady state), while lower activity is observed in the presence of less cytoplasmic ATP (as in the uncoupled steady state), indicating that the observed inhibition (20) of phosphofructokinase by excess ATP does not completely determine its behavior. Since other adenine nucleotides act as activators (29, 30), a mechanism assuming both activation and inhibition was constructed. This is the minimal mechanism that enabled the model to reproduce the experimental results to be duplicated, and is almost certainly incomplete (partly owing to computer memory limitations) and possibly incorrect as well. This assumes a substrate activation (so that the enzyme is relatively inactive at low concentrations of fructose 6-phosphate, but activity increases strongly as fructose 6-phosphate concentration increases); also that the enzyme is activated by ADP in addition to its activation by fructose phosphate; and that it is product-inhibited (the complex of magnesium ion with fructose diphosphate (31) may be involved as well as fructose diphosphate itself).

Almost immediately after this model of phosphofructokinase had been completed, the experimental work of Passonneau and Lowry (21, 32) became available, showing that there is indeed an activation by the substances assumed (ADP' and fructose 6-phosphate) as well as by inorganic phosphate. The principal disagreement is that Passonneau and Lowry found that fructose diphosphate is an activator at low concentrations. It seems unlikely that fructose diphosphate could be equally effective at the high concentrations which were observed by Hess (19); otherwise the observed fructose 6-phosphate concentrations could not be simultaneously present at all. The product inhibition postulated here results in low activity in the glucose-saturated steady state when the concentration of fructose diphosphate is high and that of cytoplasmic ATP is low. ADP activation accounts for the high activity of this enzyme during the initial transients after glucose addition, as well as when an uncoupling agent is added. Substances such as AMP and cyclic adenosine 3', 5'-monophosphate, for which no experimental concentrations are available, have not been considered in this model.

Aldolase—The mechanism for aldolase was largely as indicated by the work of Richards and Rutter (33-35) and of Rose and Rieder (36, 37). It is assumed (Equations 22 through 27, Table 1) that this enzyme forms a fairly stable complex with dihydroxyacetone phosphate; also (see below) that it combines with triosephosphate isomerase.

Triosephosphate Isomerase—The mechanism of triosephosphate isomerase is not too well known. Rose et al. have shown (38) that the reaction catalyzed by this enzyme is not in equilibrium in rat liver, and an interesting anomaly is observed in ascites cells in connection with this enzyme: an equilibrium mixture of dihydroxyacetone phosphate and glyceraldehyde phosphate is 96% dihydroxyacetone phosphate, whereas in the cells there is more glyceraldehyde phosphate than dihydroxyacetone phosphate. However, this enzyme is apparently converting dihydroxyacetone phosphate to glyceraldehyde phosphate (since the main glycolytic pathway goes through glyceraldehyde phosphate and dihydroxyacetone phosphate is effectively on a branch of it). In other words, it is apparently catalyzing a reaction in a direction away from equilibrium. Checks of the experimental data indicate no gross error, and the permeability properties of phosphorylated trioses indicate that this observed anomaly is not due to a compartmentation effect.

The mechanism of triosephosphate isomerase itself has been described in the simplest possible terms; but to account for the anomaly it has been necessary to postulate that this enzyme is bound to aldolase. The two enzymes were originally supposed to be one and the same (39), but no physiological role has hitherto been suggested for the combination of these enzymes. This combination is such as to result in a high local concentration of dihydroxyacetone phosphate at the active center of triosephosphate isomerase. This mechanism has been described in detail elsewhere (2). In order to make this mechanism fit all the available data, it has also been necessary to postulate that the extent of this binding is to some degree controlled by a glycolytic intermediate which is present in larger amount in the glucose-saturated steady state than in the endogenous steady state. We have chosen dihydroxyacetone phosphate for convenience, but there is no evidence that this is the intermediate involved. A somewhat similar control mechanism has been noted by Wu and Racker, who found phosphofructokinase to be limiting in glycolysis in ascites cell extracts where the protein concentration was low, but not where it was high (12).

Glyceraldehyde Phosphate Dehydrogenase—The mechanism for glyceraldehyde phosphate dehydrogenase was taken from the previous model (4), with some modification of the rate constants. It is assumed here that all inorganic phosphate present in the cell is available to this enzyme and that phosphate otherwise neither activates nor inhibits it. As some evidence in favor of a phosphate compartmentation has been found by Wu and Racker (13), this may be oversimplified.

Mutases and Kinases—The mechanisms for phosphoglyceromutase (28), phosphoglycerokinase (28), enolase (40, 41), and pyruvate kinase (42) were obtained from the literature. Enolase is assumed to be inhibited by 3-phosphoglyceric acid (41), and pyruvate kinase has been shown to be inhibited by ATP (42). These models are simpler than those of the enzymes described above because the detailed transient kinetics needed for elaboration of mechanisms is not available for most of their products and substrates.

Lactic Dehydrogenase—The mechanism used for lactic dehydrogenase is the compulsory order binding mechanism of Winer and Schwert (43). It was assumed on the basis of experiments by Bücher and Klingenberg (44) that the amount of this enzyme is capable of converting 600 \( \mu \) moles of pyruvate to lactate per second per kg of cells, wet weight; but the amount was varied, as described below. Here, again, no assumptions were made as to inhibitors or activators, even though for some forms of lactic dehydrogenase these have been shown to exist (45-47).

Oxidative Phosphorylation—The mechanism for oxidative phosphorylation and mitochondrial regulation of metabolism was taken almost intact from the previous model (4). This includes compartmentation of ATP and DPN or DPNH between the mitochondria and the cytoplasm, and an oxidative phosphorylation mechanism in terms of hypothetical intermediates, X1, XSL, and XSP. The principal change required was the assumption that some hydrolysis of mitochondrial ATP takes place; otherwise it would be impossible to maintain an ap-
preciable level of free ADP when nearly all the adenine nucleotide
is in the mitochondria as ATP (the previous model (4) did not
provide for such free ADP).
Also included in the model were a number of minor interactions
such as metabolic utilization of cytoplasmic ATP (this may
alternatively be considered as cytoplasmic ATPase). These
were generally taken from the previous model (4).

**Performance of Model**

The following behavior would be expected of the model, according
to the known kinetic behavior of ascites cells.

1. The observed endogenous and glucose-inhibited steady
states should be maintained.
2. Oxygen consumption should in general be about one-third
of the glucose uptake, which is initially 300 μmoles per kg of
cells, wet weight, per second; and in the glucose-saturated steady
state, 27.2 μmoles per kg of cells, wet weight, per second.

**Graph 1.** Hexoses and hexose phosphates

**Graph 2.** Adenine nucleotides

**Graph 3.** Triose phosphates

**Graph 4.** Three carbon acids (except pyruvate and lactate)

**Graph 5.** DPN, DPNH, and pyruvate

**Graph 6.** Oxygen and oxidative phosphorylation intermediates

![Graph 1](image1)
![Graph 2](image2)
![Graph 3](image3)
![Graph 4](image4)
![Graph 5](image5)
![Graph 6](image6)
3. On the addition of glucose, the following transient changes are expected: glucose 6-phosphate should rise to a peak (about 40% of the maximum) at about 10 seconds, fall to a minimum (about 30% of the maximum) near 30 seconds, then rise to its maximal value (11, 14).

Fructose 6-phosphate does not undergo much change in concentration during the first minute, although there may be a slight peak (15).

Fructose 1,6-diphosphate builds up to a maximum in about 1 minute, reaching 40% of this in 15 seconds (11).

Nearly all the trioses and their carbon compounds build up more slowly; few marked transients have been observed. Lemberg-Holm (15, 16) observed a slight peak for 3-phosphoglyceric acid relatively early in the transient state at 37°C; a similar (although much slighter) peak has been found in this model. Probably this difference is due to the fact that aldolase is the limiting enzyme of this model during initial transients (its energy of activation has been shown to be quite high (34)), but is not limiting at 37°C.

4. On the addition of an uncoupling agent to the glucose-inhibited state, the following transient changes are expected: the rate of uptake of both glucose and oxygen should increase several times; lactate builds up, and often pyruvate does so also (11), but exceptions have been noted (19).

This was indeed the performance of the model, as shown in Figs. 2 and 3, after corrections had been made to insure a sufficient supply of some reactants that are used up in the course of glycolysis. For example, a literal interpretation of Table II would indicate that only the oxygen actually present within the ascites cells is considered in the model. When the computation was actually performed in this way, the oxygen was promptly exhausted. Accordingly, the oxygen was treated as if it were also present in a suspension volume 10 times as great as that of the cells, which leads to satisfactory agreement with experiment.

Graph 2. Three carbon acids, DPN, DPNH, and glyceraldehyde 3-phosphate.

Graph 3. Oxygen, mitochondrial DPNH, and various intermediates.

Graph 4. Rate of glucose uptake and concentration of DPNH as a function of inorganic phosphate concentration, during the course of the calculations.
The same problem arises with phosphate: the build-up of phospho-
phorylated glycolytic intermediates is greater than the amount of
phosphate initially present. The hypothetical experiment
(which probably has no natural counterpart) was carried out of
allowing inorganic phosphate to become almost completely ex-
hausted. At this point, the metabolism of glyceraldehyde 3-phosphate
had nearly stopped and the DPNH level had fallen greatly. Fig. 4 shows the
relationship of DPNH concentration to phosphate concentration in
the course of this experiment, and for comparison the rate of glu-
ose uptake (which is relatively unaltered). In order to permit
the computation to be continued, the inorganic phosphate con-
centration was reset to its starting value and held constant there-
after (by suitable manipulation in the program). These two
modes of computation correspond to phosphate being present in
the same volume as the other glycolytic intermediates and to
its being present in a much larger volume, respectively. The be-
havior of phosphate is undoubtedly more complex than could be
properly represented in the model, e.g., it has been suggested (13,
19) that there may be compartmentation of inorganic phosphate so
that only a part is available for glycolysis, and that the rate at
which phosphate enters the cell may be limiting. The computa-
tions have been carried out with the simplest possible approxima-
tion for phosphate behavior, which we hope to study more thor-
oughly in the future.

The results of computation in Fig. 2 include the following three
situations: (a) phosphate was treated like any other substance,
and being exhausted; (b) the phosphate level had been restored
to its original level and held constant thereafter; and (c) this
second result was corrected approximately, by hand, for the ap-
parent synthesis of ADP described under "Method." It will be
seen that the result corrected for both phosphate and ADP is in
good agreement with the available experimental data.

A detailed and quantitative comparison of the behavior of the
model with that observed experimentally is given in Tables IV to
VI. Table IV shows a comparison of the concentration values
for the computer representation and the ascites cells themselves.
It is seen that in nearly all cases the concentrations are in very
close agreement; the only appreciable deviations are for glucose
6-phosphate and for pyruvate in the uncoupled state. The glu-
cose 6-phosphate discrepancy may be due to the fact that it was
not possible to include magnesium ions in the mechanism for hexo-
kinase. Whether the pyruvate difference actually represents a
discrepancy is questionable: in one similar cell preparation (19)
the amount of pyruvate did decrease when an uncoupler was
added, but a number of other preparations have been observed in
which adding an uncoupler increased both the amount of pyru-
vate and the pyruvate to lactate ratio (11).

Concentration ratios are shown in Table V at the same points
in the computer solution and the experiments as for Table IV.
Here again the agreement between the values obtained experi-
mentally and those computed is quite satisfactory with but two
exceptions: the lactate to pyruvate ratio (discussed above) and
the ratio of fructose 6-phosphate to fructose 1,6-diphosphate in
the uncoupled state. The latter may be due to possible inade-
quacies of the model for phosphofructokinase. In the cases of
both concentrations and ratios of concentrations, the accuracy of
the representation has been improved appreciably over the previ-
ous model (4).

In considering the ratio of concentrations for DPNH, it is

<table>
<thead>
<tr>
<th>Substance</th>
<th>Endogenous just before adding glucose</th>
<th>Glucose-activated at glucose 6-phosphate peak</th>
<th>Glucose-inhibited just before adding uncoupler</th>
<th>Uncoupled at right end of Fig. 3</th>
</tr>
</thead>
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<tr>
<td></td>
<td>Computer</td>
<td>Cells*</td>
<td>Computer</td>
<td>Cells†</td>
</tr>
<tr>
<td>Lactate</td>
<td>6.7</td>
<td>6.58</td>
<td>6.9</td>
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<tr>
<td>Pyruvate</td>
<td>0.044</td>
<td>0.04</td>
<td>0.05</td>
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</tr>
<tr>
<td>Glucose 6-phosphate</td>
<td>0.13</td>
<td>0.10</td>
<td>0.9</td>
<td>ca. 0.9</td>
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<tr>
<td>Fructose 6-phosphate</td>
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<td>0.05</td>
<td>0.06</td>
<td>Small</td>
</tr>
<tr>
<td>Fructose 1,6-diphosphate</td>
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<td>0.15</td>
<td>0.8</td>
<td>ca. 0.8</td>
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<tr>
<td>α-Glycerophosphate</td>
<td>1.12</td>
<td>1.09</td>
<td>1.18</td>
<td></td>
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<td>Dihydroxyacetone phosphate</td>
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<td>0.12</td>
<td>0.165</td>
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<tr>
<td>Glyceraldehyde phosphate</td>
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<td>0.17</td>
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<tr>
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<td>0.02</td>
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<td>0.15</td>
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<tr>
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<td>0.164</td>
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<tr>
<td>DPN (mitochondrial)</td>
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<td>DPNII (cytoplasmic)</td>
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<tr>
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<td>ADP</td>
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<td>3.06</td>
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</table>

* From (19).
† Estimated from (11), Fig. 5.
‡ Estimated from the changes on adding dicoumarol in another preparation reported in (19).
The first of these assumptions is simply that the enzymes within of the model, implicitly or explicitly, which are of some interest. The enzymes in the ascites cell act in much the same way as they have been observed to act in the test tube. The fact that this assumption yields satisfactory results indicates that for the glycolytic pathway it is unlikely that the initial substrate may be bound to an enzyme, transformed into part of an activated complex, and then carried through the rest of the pathway in the form of this complex.

It has been explicitly assumed that ATP and the DPN-DPNH couple are compartmented between the cytoplasm and mitochondria. In previous models this yielded qualitatively correct results (4); it can now be seen to yield quantitatively correct results as well. There is also considerable experimental evidence in favor of this assumption. It has not yet been possible to construct and study the behavior of models which do not include one or both of these assumptions, although it is possible that such models might represent the data equally well.

Another assumption, which may be considered to be made up of many pieces of information from the existing literature, is that many of the enzymes of the glycolytic pathway are regulated by other enzymes in the pathway by way of inhibitions or activations by substrates or products. Sufficient experimental information is not yet available to determine the generality of this phenomenon. In this way, the glycolytic pathway seems to contrast strongly with certain pathways of amino acid metabolism (for instance) where the first enzyme is strongly inhibited by the final product.

It has been possible to carry out a number of experiments with the model. It has been proposed that the relative ratios of DPNH and DPN are determined primarily by lactic dehydrogenase (48, 49) and that these two are normally in equilibrium with lactic dehydrogenase equilibrium. The closest approach of this phenomenon. In this way, the glycolytic pathway seems to contrast strongly with certain pathways of amino acid metabolism (for instance) where the first enzyme is strongly inhibited by the final product.

A number of assumptions have been made in the construction of the model, implicitly or explicitly, which are of some interest. The first of these assumptions is simply that the enzymes within the ascites cell act in much the same way as they have been observed to act in the test tube. The fact that this assumption yields satisfactory results indicates that for the glycolytic pathway it is unlikely that the initial substrate may be bound to an enzyme, transformed into part of an activated complex, and then carried through the rest of the pathway in the form of this complex.

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It has been possible to carry out a number of experiments with the model. It has been proposed that the relative ratios of DPNH and DPN are determined primarily by lactic dehydrogenase (48, 49) and that these two are normally in equilibrium with lactic dehydrogenase equilibrium. The closest approach of DPNH:DPN equilibrium was in the glucose-saturated steady state. In the course of checking out this finding, the concentration of lactic dehydrogenase included in the model was increased by a factor of 10 (with no changes in mechanism, rate constants, yields satisfactory results indicates that for the glycolytic pathway it is unlikely that the initial substrate may be bound to an enzyme, transformed into part of an activated complex, and then carried through the rest of the pathway in the form of this complex.

It has been explicitly assumed that ATP and the DPN-DPNH couple are compartmented between the cytoplasm and mitochondria. In previous models this yielded qualitatively correct results (4); it can now be seen to yield quantitatively correct results as well. There is also considerable experimental evidence in favor of this assumption. It has not yet been possible to construct and study the behavior of models which do not include one or both of these assumptions, although it is possible that such models might represent the data equally well.

Another assumption, which may be considered to be made up of many pieces of information from the existing literature, is that many of the enzymes of the glycolytic pathway are regulated by other enzymes in the pathway by way of inhibitions or activations by substrates or products. Sufficient experimental information is not yet available to determine the generality of this phenomenon. In this way, the glycolytic pathway seems to contrast strongly with certain pathways of amino acid metabolism (for instance) where the first enzyme is strongly inhibited by the final product.

It has been possible to carry out a number of experiments with the model. It has been proposed that the relative ratios of DPNH and DPN are determined primarily by lactic dehydrogenase (48, 49) and that these two are normally in equilibrium with lactic dehydrogenase equilibrium. The closest approach of DPNH:DPN equilibrium was in the glucose-saturated steady state. In the course of checking out this finding, the concentration of lactic dehydrogenase included in the model was increased by a factor of 10 (with no changes in mechanism, rate constants,
etc.); although this did reduce the ratio of DPNH to DPN, it was still not sufficient to make this ratio what one would calculate from the lactic dehydrogenase equilibrium, and did not drastically change the behavior of the model. The experiment with varying phosphate concentration in this model indicates that the enzyme which most strongly influences the ratio of DPNH to DPN is glyceraldehyde 3-phosphate dehydrogenase, i.e., the enzyme that makes DPNH from DPN rather than the one that makes DPN from DPNH. The other enzyme of the pathway which interconverts DPN and DPNH, α-glycerophosphate dehydrogenase, is of relatively little importance in ascites cells.

The rate of glycolysis (measured by glucose uptake (Fig. 4) and the formation of some intermediates along the chain (Fig. 2)) does not depend greatly on phosphate concentration for the short periods of time studied here, although the ratio of DPNH to DPN in the system does depend strongly on the amount of phosphate, which is not surprising since DPNH is primarily generated in a reaction requiring phosphate. Undoubtedly the system would not continue to glycolyze at the same rate over longer periods of time in the absence of phosphate, but our short time "experiments" tentatively indicate that adenine nucleotides are more important in control than phosphate. This conclusion depends heavily on the assumption that phosphate primarily interacts with glyceraldehyde 3-phosphate dehydrogenase as required by the stoichiometric equations, and that it is relatively unimportant in activating or inhibiting enzymes. Since such enzymes as phosphofructokinase are activated by phosphate (21), this subject is being explored further.

It has been repeatedly noted that owing to the limited memory capacity of the computer used, and also the limited knowledge about some of the enzymes in the pathway, this model is incomplete. Important deficiencies are the inability to include hydrogen ion and magnesium ion, and the effects of various kinases (such as myokinase) and nucleotides other than adenosine di-phosphate and triphosphate. Also deserving of further study are alternate pathways, such as the pentose shunt and the synthesis of ribonucleic acid, and the effects of repression. Of all the above, the omission of hydrogen and magnesium ion is probably the most important, as many of the reactions involved are dependent on both of these. It is hoped to carry these experiments further on this model or its successors, with the assistance of more powerful computers.

**SUMMARY**

1. A detailed computer model of the glycolytic and respiratory pathway of Ehrlich ascites tumor cells has been constructed, consisting of 89 reactions among 65 chemicals (enzymes, substrates, complexes, etc.). The model is based on observed steady state concentrations of all glycolytic intermediates and over-all fluxes from one specific cell preparation, supplemented by data from previous studies and the literature. The model quantitatively reproduces much of the aerobic glycolytic behavior of these cells.

2. The model is based on the following assumptions, some of which were made in previous work on the subject. Enzymes in vivo act as they do in vitro, so that enzymatic mechanisms determined in vitro can be directly inserted into the model. Many of the enzymes are activated or inhibited, especially by the products of other enzymes, but there is no single controlling point of feedback inhibition, as is commonly found in biosynthetic pathways. Mass-action kinetics has been followed throughout; in particular, inorganic phosphate has not been assumed to control the rate of reactions, other than the ordinary mass-action law control of reactions in which it is consumed. Diphosphopyridine nucleotide and its reduced form and adenosine triphosphate are compartmented between the cytoplasm and the mitochondria. Phosphofructokinase has a complex pattern of activation and inhibition. Aldolase is the limiting enzyme at the temperature (22°) being simulated. The mechanism of yeast hexokinase has been incorporated into the model.

3. The differential equations corresponding to the chemical equations composing the model are written by the computer and solved by a modified Euler point-slope method, with the step size regulated to keep truncation error at 1%. The model is mathematically ill behaved, and a number of expedients have been developed to permit economical computation.

4. In the course of constructing the model, it was found possible to obtain information as to enzymatic mechanism (primarily under transient rather than steady state conditions), especially for hexokinase, phosphofructokinase, and aldolase-triosephosphate isomerase.

5. The adenine nucleotides are found to be the most important controlling factors in the model.

6. The behavior of the model was not in accord with the suggestion of Büchler that lactic dehydrogenase keeps the DPN-DPNH system of the cytoplasm in equilibrium with lactate and pyruvate. Glyceraldehyde 3-phosphate dehydrogenase strongly influenced the DPN-DPNH system, with appropriate dependence on the inorganic phosphate level.

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