Simulation of the Pentose Cycle in Lactating Rat Mammary Gland

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A computer model representing the pentose cycle, the tricarboxylic acid cycle and glycolysis in slices of lactating rat mammary glands has been constructed. This model is based primarily on the studies, with radioactive chemicals, of Abraham & Chaikoff (1959) [although some of the discrepant data of Katz & Wals (1972) could be accommodated by changing one enzyme activity]. Data obtained by using [1-¹⁴C]-, [6-¹⁴C]- and [3,4-¹⁴C]-glucose were simulated, as well as data obtained by using unlabelled glucose (for which some new experimental data are presented). Much past work on the pentose cycle has been mainly concerned with the division of glucose flow between the pentose cycle and glycolysis, and has relied on the assumption that the system is in steady state (both labelled and unlabelled). This assumption may not apply to lactating rat mammary glands, since the model shows that the percentage flow through the shunt progressively decreased for the first 2 h of a 3 h experiment, and we were unable to construct a completely steady-state model. The model allows examination of many quantitative features of the system, especially the amount of material passing through key enzymes, some of which appear to be regulated by NADP⁺ concentrations as proposed by McLean (1960). Supplementary information for this paper has been deposited as Supplementary Publication SUP 50023 at the British Museum (Lending Division) (formerly the National Lending Library for Science and Technology), Boston Spa, Yorks. LS23 7BQ, U.K., from whom copies can be obtained on the terms indicated in *Biochem. J.* (1973) 131, 5.

The role of the pentose cycle in glucose catabolism has been evaluated by two general methods; (1) by measurement of the activities of the individual enzymes, and (2) by determination of the relative contribution of the pathway by measuring the labelled products from specifically labelled precursors. The lactating rat mammary gland has been studied extensively by both methods. The pentose-cycle activity has been demonstrated here by observing high activities of glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase (Glock & McLean, 1954; McLean, 1958*a,b*; Baldwin & Milligan, 1966; Novello & McLean, 1968; Gul & Dils, 1969), by the isolation of pentose-cycle intermediates (sedoheptulose 7-phosphate and pantoic phosphate by Peeters *et al.*, 1957; 6-phosphogluconate, pantoic phosphate, sedoheptulose 7-phosphate and erythrose 4-phosphate by Gumaa *et al.*, 1971), and by the demonstration of transketolase and transaldolase activity (Novello & McLean, 1968; Gumaa *et al.*, 1971).

There are problems associated with the interpretation of these data. Enzyme activities, such as those measured by Baldwin & Milligan (1966), Novello &

McLean (1968) or Gumaa *et al.* (1971), are normally measured at close-to-optimal conditions, which may be quite different from physiological conditions. The flux through an enzyme *in situ* depends not only on the concentrations of reactants and modifiers, but also on compartmentation, binding of enzymes to membranes and other proteins, and interaction between enzymes that may cause apparent deviations from their behaviour *in vitro* (Garfinkel *et al.*, 1968). Other sources of difficulty in interpretation are: several substances (glucose 6-phosphate, fructose 6-phosphate and glyceraldehyde 3-phosphate) are intermediates in both the pentose cycle and glycolysis, randomization is introduced by the transaldolase and transketolase reactions, and exchange of radioactive label without net flux can occur (Heath, 1968).

Considerable work has been done, with pencil and paper rather than computer methods, in analysing relative pathway contributions at steady state (Wood *et al.*, 1963; Katz & Wood, 1963; Landau *et al.*, 1964; Katz *et al.*, 1966; Landau & Bartsch, 1966; Landau *et al.*, 1966; Katz & Rognstad, 1967). In these analyses, equations have been derived for incorporation of radioactive label from a specifically labelled precursor (e.g. [1-¹⁴C]glucose) into specific products (e.g. ¹⁴CO₂ or [¹⁴C]lactate), or into specific carbon atoms of a product (e.g. C-1, C-2 etc., of a hexose).

The present paper describes a set of computer

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models that permit study of the factors controlling the relationship between the pentose cycle and other catabolic processes under both non-steady-state and steady-state conditions. Important advantages of these simulations in evaluating pathway contributions are: (1) they permit data obtained by different experimental methods on a given system (studies with labelled compounds, measurement of enzyme activities, determination of substrate concentrations) to be combined readily; (2) they facilitate study of the relation between substrate fluxes and isotopic equilibrium; and (3) they allow the use of data from tissues undergoing changes in metabolism caused by cell death, changes in permeability owing to cation losses (Flink *et al.*, 1950), substrate-level alterations (Katz & Wood, 1963) etc. Such changes may be significant when incubation times of hours are used.

Methodology

The models were constructed with the Control Data 160-A, Digital Equipment PDP-6 and PDP-10 computers, located at the University of Pennsylvania Medical School Computer Facility. The simulation language of Garfinkel (1968) and its predecessors (Garfinkel *et al.*, 1961; Garfinkel & Hess, 1964) were used to formulate the models, following the approach of Garfinkel (1966*b*, 1970) in simulating use of radioactive tracers in brain metabolism.

Matching models approximately describing the behaviour of the biochemical system containing labelled compounds and the system with appropriate labelled inputs are constructed, and their behaviour is compared with available experimental data. As the 'unlabelled' model is usually much smaller than the 'labelled' models, most of the manipulation is carried

out with it, and the resulting rate constants etc. are transposed to the 'labelled' models. Rate constants are initially approximated from equilibrium constants and K_m values for the enzymes involved (although the enzyme representations here are too crude to have real K_m values), and steady-state concentrations or initial concentrations and net fluxes are approximated from data obtained by using labelled and unlabelled compounds. The chemical equations and kinetic parameters are repeatedly modified until the entire set of models closely approximates to as much of the available experimental data as possible.

Three 'labelled' models were constructed, representing the results of adding [1- ^{14}C]glucose, [6- ^{14}C]glucose, or [3,4- ^{14}C]glucose to slices of lactating rat mammary gland. Equations for these models were written by using a program (Haut & Basickes, 1967) that determines the labelling pattern of all metabolites after addition of a specifically labelled substrate to a specific unlabelled system.

As a crude first approximation, flux through the 6-phosphogluconate dehydrogenase reaction was set equal to the production of $^{14}\text{CO}_2$ from [1- ^{14}C]glucose; flux through the tricarboxylic acid cycle was set equal to twice the rate of production of $^{14}\text{CO}_2$ from [6- ^{14}C]glucose (see below); flux through the pyruvate dehydrogenase reaction was set equal to the rate of production of $^{14}\text{CO}_2$ from [3,4- ^{14}C]glucose; flux through the phosphoglucose isomerase reaction was set so as to fit the production of ^{14}C -labelled fatty acid from [6- ^{14}C]glucose. More precise estimation of the contributions of the shunt, the tricarboxylic acid cycle and the pyruvate dehydrogenase reaction were then obtained with the [1- ^{14}C]glucose, the [6- ^{14}C]glucose and the [3,4- ^{14}C]glucose models respectively.

Table 1. Comparison of observed and calculated ^{14}C -labelling of CO_2 and fatty acids (in percentages of ^{14}C administered)

	The observed data were obtained from Abraham <i>et al.</i> (1957).			
	Label incorporated into $^{14}\text{CO}_2$ (% of ^{14}C administered)		Label incorporated into ^{14}C -labelled fatty acids (% of ^{14}C administered)	
	Obs.	Calc.	Obs.	Calc.
[1- ^{14}C]Glucose				
1 h	10.2	9.9	2.9	3.1
2 h	14.9	15.6	4.3	4.3
3 h	19.7	19.7	5.7	4.8
[6- ^{14}C]Glucose				
1 h	0.8	0.4	8.2	5.6
2 h	2.8	1.4	11.1	7.7
3 h	3.4	2.2	14.1	8.7
[3,4- ^{14}C]Glucose				
3 h*	13.4	15.9	1.5	1.1

* This is the only time-point available for this ^{14}C -labelling in this set of data. In another study (Abraham & Chaikoff, 1959) the $^{14}\text{CO}_2$ recovery increased approximately linearly with time (although to a different final value). The values calculated here have the same curve shape.

Table 2. Comparison of observed and calculated concentrations of selected metabolic intermediates

The concentrations were measured by Dr. Rene Frenkel under conditions identical with those in the radioisotope experiments referred to in Table 1. Concentrations are expressed in $\mu\text{mol}/0.5\text{g}$ wet wt. of tissue.

Time (min)	Glucose 6-phosphate		Fructose 6-phosphate		Fructose 1,6-diphosphate		Dihydroxyacetone 3-phosphate		Glyceraldehyde 3-phosphate	
	Obs.	Calc.	Obs.	Calc.	Obs.	Calc.	Obs.	Calc.	Obs.	Calc.
0	0.092 0.024 (0.094)*	0.058	0.047 0.016 (0.031)*	0.031	0.007 0.018 (0.026)*	0.013	0.045 0.026 (0.040)*	0.035	0.017 0.004 (0.004)*	0.010
20	0.131 0.095	0.176	0.040 0.032	0.053	0.003 0.003	0.013	0.070 0.071	0.101	0.032 0.038	0.038
40	0.191 0.256	0.198	0.043 0.056	0.052	0.012 0.004	0.013	0.060 0.113	0.100	0.025 0.056	0.038
60	0.189 0.162	0.213	0.056 0.044	0.049	0.024 0.005	0.012	0.079 0.057	0.092	0.048 0.014	0.035
120	0.167 0.187	0.207	0.042 0.051	0.041	0.009 0.005	0.010	0.107 0.118	0.075	0.055 0.062	0.028
180	0.159 0.154	0.181	0.038 0.053	0.036	0.007	0.009	0.088 0.070	0.065	0.043 0.014	0.025

*Values in parentheses are from Gumaa *et al.* (1971).

Data base for the model

Initially, the models were based on the data, obtained by using labelled material, of Abraham *et al.* (1957), the enzymological data of McLean (1958a,b), Baldwin & Milligan (1966) and Novello & McLean (1968), and substrate measurements made by Dr. R. A. Frenkel (personal communication). The model was then changed to reflect some of the more recent data (e.g. Gumaa *et al.*, 1971; Katz & Wals, 1972).

The data, obtained by using labelled materials, of Abraham *et al.* (1957) were used as the basis of the model. These workers reported on the percentage of added ^{14}C recovered as $^{14}\text{CO}_2$ and as ^{14}C -labelled fatty acids after 1, 2, and 3 h incubation of slices (500mg) of lactating rat mammary gland (10–14 days *post partum*) in 5ml of Krebs–Henseleit (1932) buffer, containing 50 μmol of glucose labelled with ^{14}C in C-1, C-6, or C-3 and C-4. A striking aspect of their results, summarized in Table 1, is the significantly decreased rate of production of $^{14}\text{CO}_2$ from $[1\text{-}^{14}\text{C}]$ glucose during the second and third hours, and of production of ^{14}C -labelled fatty acid from both $[1\text{-}^{14}\text{C}]$ - and $[6\text{-}^{14}\text{C}]$ glucose. Production of $^{14}\text{CO}_2$ and ^{14}C -labelled fatty acid from $[3,4\text{-}^{14}\text{C}]$ glucose after a 3 h incubation period is reported by Abraham *et al.* (1957), but there is some question about the validity of these results. Whereas Abraham *et al.* (1957) found the $^{14}\text{CO}_2$ produced to be about two-thirds of that produced from $[1\text{-}^{14}\text{C}]$ glucose, Katz & Wals (1972; personal communication) found that the yield of $^{14}\text{CO}_2$ from $[3,4\text{-}^{14}\text{C}]$ glucose (and from $[3\text{-}^{14}\text{C}]$ glucose) exceeded that from $[1\text{-}^{14}\text{C}]$ glucose. Alterations

in the model necessary to accommodate these divergent results are discussed below.

Substrate concentrations were measured by Dr. R. A. Frenkel (Table 2) on tissue slices incubated exactly as in the study of Abraham *et al.* (1957) with samples frozen at specific intervals.

Construction of the model

The overall structure of the model is given in Scheme 1. The computer input used to define the 'unlabelled' model is given in Table 3.

The decrease in $^{14}\text{CO}_2$ production from $[1\text{-}^{14}\text{C}]$ -glucose during the second and third hours may have two mechanisms: (1) decrease in the amount of glucose 6-phosphate available for conversion into ribulose 5-phosphate and CO_2 by glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase by inhibition of hexokinase, and (2) inhibition of one or both of these dehydrogenases. To make the model fit all available experimental data, it was necessary to incorporate both. Control by glucose 6-phosphate availability is involved in the uniform decreases in both pentose cycle and glycolysis during the second and third hours. Abraham *et al.* (1957) showed a proportional decrease in the production of $^{14}\text{CO}_2$ and of ^{14}C -labelled fatty acid from $[1\text{-}^{14}\text{C}]$ -glucose. Since ^{14}C -labelled fatty acid is produced from products of glycolysis, this indicates inhibition of glycolysis. However, the decrease in $^{14}\text{CO}_2$ production from $[1\text{-}^{14}\text{C}]$ glucose also requires control by $\text{NADP}^+/\text{NADPH}$. Without this control, the decrease in production of CO_2 in the shunt during the second

Table 3. *Statement of the 'unlabelled' model*

The counterparts for ^{14}C -labelling have been deposited as Supplementary Publication SUP50023 at the British Museum (Lending Division) (formerly the National Lending Library for Science and Technology). Numbers in parentheses are starting concentrations ($\mu\text{mol}/0.5\text{ g wet wt. of tissue}$); where none is specified a value of 10^{-9} was automatically set.

Explanation and reaction	Rate constant(s)*
(50.0) Glucose (outside cell) $\rightleftharpoons$ glucose (inside cell)	0.050, 0.886
Glucose $\rightarrow$ lactose	0.009
Glucose + (0.001) hexokinase $\rightleftharpoons$ glucose-hexokinase	378.0, 37.8
Glucose-hexokinase $\rightarrow$ (0.058) glucose 6-phosphate + (0.001) hexokinase	1000
(0.058) Glucose 6-phosphate + (0.001) hexokinase $\rightleftharpoons$ hexokinase (inhibited)	1.667, 0.100
Phosphoglucose isomerase: (0.058) glucose 6-phosphate $\rightleftharpoons$ (0.0312) fructose 6-phosphate	0.360, 0.010
Phosphofructokinase: (0.0312) fructose 6-phosphate $\rightleftharpoons$ (0.0125) fructose 1,6-diphosphate	2.500, 0.300
Aldolase: (0.0125) fructose-1,6-diphosphate $\rightleftharpoons$ (0.010) glyceraldehyde 3-phosphate + (0.0352) dihydroxyacetone 3-phosphate	10.0, 0.442
Triose phosphate isomerase: (0.0352) dihydroxyacetone 3-phosphate $\rightleftharpoons$ (0.010) glyceraldehyde 3-phosphate	30.0, 75.6
(0.0352) Dihydroxyacetone 3-phosphate $\rightleftharpoons$ (0.437) glycerol	225, 50
(0.01) Glyceraldehyde 3-phosphate $\rightarrow$ (0.059) pyruvate	7.5
Glucose 6-phosphate dehydrogenase: (0.058) glucose 6-phosphate + (0.019) NADP^+ $\rightleftharpoons$ (0.094) 6-phosphogluconolactone + (3.8) NADPH	35.0, 2.07
6-Phosphogluconolactonase: (0.094) 6-phosphogluconolactone $\rightleftharpoons$ (0.094) 6-phosphogluconate	0.191, 0.0191
6-Phosphogluconate dehydrogenase: (0.094) 6-phosphogluconate + (0.019) NADP^+ $\rightleftharpoons$ (0.5) ribulose 5-phosphate + CO_2A + (3.8) NADPH	17.2, 1.0
Phosphoribose isomerase: (0.5) ribulose 5-phosphate $\rightleftharpoons$ (0.5) ribose 5-phosphate	0.05, 0.0162
D-Ribose 5-phosphate 3-epimerase: (0.5) ribulose 5-phosphate $\rightleftharpoons$ (0.5) xylulose 5-phosphate	0.102, 0.069
Transketolase: (0.5) ribose 5-phosphate + (0.5) xylulose 5-phosphate $\rightleftharpoons$ (0.06) sedoheptulose 7-phosphate + (0.01) glyceraldehyde 3-phosphate	0.72, 0.60
(0.004) Erythrose 4-phosphate + (0.5) xylulose 5-phosphate $\rightleftharpoons$ (0.0312) fructose 6-phosphate + (0.01) glyceraldehyde 3-phosphate	28.5, 2.75
Transaldolase: (0.06) sedoheptulose 7-phosphate + (0.01) glyceraldehyde 3-phosphate $\rightleftharpoons$ (0.0312) fructose 6-phosphate + (0.004) erythrose 4-phosphate	20.5, 19.5
Lactate dehydrogenase: (0.059) pyruvate $\rightleftharpoons$ (6.4) lactate	0.03, 0.0005
Pyruvate dehydrogenase: (0.059) pyruvate $\rightarrow$ (0.100) acetyl-CoA + CO_2B	1.0
CO_2 fixation: (0.059) Pyruvate + CO_2A $\rightarrow$ (0.307) CIT0C	0.05
(0.059) Pyruvate + CO_2B $\rightarrow$ (0.307) CIT0C	0.05
(0.059) Pyruvate + CO_2C $\rightarrow$ (0.307) CIT0C	0.05
(0.059) Pyruvate + CO_2D $\rightarrow$ (0.307) CIT0C	0.05
(3.8) NADPH $\rightarrow$ (0.019) NADP^+	0.015
(0.100) Acetyl-CoA + (3.8) NADPH $\rightleftharpoons$ (6.667) fatty acids + (0.019) NADP^+	0.78, 0.000112
Tricarboxylic acid cycle: (0.100) acetyl-CoA + (0.307) CIT0C $\rightleftharpoons$ (0.090) CIT2C	1.7, 0.5
(0.090) CIT2C $\rightarrow$ (0.017) CIT1C + CO_2C	0.375
(0.017) CIT1C $\rightarrow$ (0.307) CIT0C + CO_2D	0.375

* If two constants are given, the first is for the forward direction.

and third hours would require a corresponding decrease in glucose 6-phosphate to concentrations far below those measured by Dr. R. Frenkel (Table 2). With this control, it is the falling concentration of NADP^+ that lowers the fluxes through glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase.

Glucose entry into the cell and hexokinase (EC 2.7.1.1)

The amount of available glucose 6-phosphate could be decreased during the second and third hours either by diminished entry of glucose into the cell or by the known product-inhibition of hexokinase.

There is insufficient evidence available to discriminate between these. Since both affect the model similarly, hexokinase inhibition was arbitrarily assigned the predominant role.

Glucose 6-phosphate dehydrogenase (EC 1.1.1.49), 6-phosphogluconolactonase (EC 3.1.1.31) and 6-phosphogluconate dehydrogenase (EC 1.1.1.44)

These three enzymes were each represented by a single reaction. Net flux through these reactions was first set equal to several times the rate of $^{14}\text{CO}_2$ production from $[1-^{14}\text{C}]\text{glucose}$ during the second and third hours; this flux diminished as the concentration

Scheme 1. Diagram of the flow of carbon through the system simulated as defined in Table 3

Reverse reactions and NADPH/NADP⁺ interconversions are not shown.

of glucose 6-phosphate and NADP⁺ available to glucose 6-phosphate dehydrogenase decreased. Rate constants for glucose entry, hexokinase, hexokinase inhibition and the dehydrogenases were then adjusted to conform to the rate of production of ¹⁴CO₂ from [1-¹⁴C]glucose in the 'labelled' model.

Representation of glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase by single equations is a crude way of representing the

dependence of their rates on substrate (glucose 6-phosphate and 6-phosphogluconic acid respectively) and NADP⁺. More sophisticated representation would have made the already large 'labelled' models (1008 equations for [3,4-¹⁴C]glucose) even more unwieldy. Whereas control at this point is necessary for the model, its exact mechanism is not critical. Glucose 6-phosphate dehydrogenase is tightly controlled by the availability of glucose 6-phosphate

(discussed above) and NADP^+ , whose concentration depends on the relative rates of its reduction and oxidation. NADP^+ is reduced to NADPH by glucose 6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase and fatty acid oxidation; NADPH is reoxidized to NADP^+ by fatty acid synthesis and 'malic' enzyme at a rate equal to the sum of their net rates. NADP^+ regeneration by 'malic' enzyme is represented by the equation:

whereas its CO_2 -fixing activity is included in the four CO_2 -fixing reactions in Table 3 (the rate for this is less than the overall rate of CO_2 fixation to pyruvate because pyruvate carboxylase also fixes CO_2).

The 6-phosphogluconolactonase reaction is sufficiently rapid to convert 6-phosphogluconolactone into 6-phosphogluconate immediately as it is formed.

Control of 6-phosphogluconate dehydrogenase is both similar and closely related to that of glucose 6-phosphate dehydrogenase. 6-Phosphogluconate availability is dependent on the rate of the glucose 6-phosphate dehydrogenase reaction; the two dehydrogenases compete for the available NADP^+ . The relative rates of reaction of these two enzymes depend on the availability of glucose 6-phosphate and 6-phosphogluconate. When there is ample glucose 6-phosphate, the glucose 6-phosphate dehydrogenase rate is somewhat faster; when the glucose 6-phosphate concentration decreases, the 6-phosphogluconate dehydrogenase rate is slightly more rapid.

In addition to decreased substrate availability, inhibition mechanisms may be important. Glaser & Brown (1955), using yeast glucose 6-phosphate dehydrogenase, have demonstrated that NADPH was a competitive inhibitor of NADP^+ (the required substrate) in the forward direction. Taketa & Pogell (1966) found that palmitoyl-CoA inhibited and inactivated both purified yeast and crude rat liver glucose 6-phosphate dehydrogenase. The inhibition was strictly competitive (and reversible for the yeast enzyme) with respect to glucose 6-phosphate, and of the mixed type with respect to NADP^+ . The large number of enzymes that palmitoyl-CoA inhibits and the apparent lack of any pattern of specificity make analysis of the role of such inhibition difficult at this time.

Phosphoglucose isomerase (EC 5.3.1.9)

This enzyme is reported as highly active in lactating rat mammary gland (Baldwin & Milligan, 1966; McLean, 1958*a,b*). However, the K_m of phosphoglucose isomerase for glucose 6-phosphate ($1 \times 10^{-3} \text{ M}$; McLean, 1958*a,b*) puts it at a competitive disadvantage against glucose 6-phosphate dehydrogenase, whose K_m for glucose 6-phosphate is $5.8 \times 10^{-5} \text{ M}$, since the glucose 6-phosphate concentration is appreciably

lower than that. The known inhibitions of phosphoglucose isomerase by shunt intermediates, specifically 6-phosphogluconate (Parr, 1965; Veitch, 1964), sedoheptulose 7-phosphate (Venkataraman & Racker, 1961) and erythrose 4-phosphate (Grazi *et al.*, 1960), will further lower its activity. For the above reasons, the rate of the phosphoglucose isomerase reaction was set as low as could be made compatible with the rate of production of ^{14}C -labelled fatty acids from $[6\text{-}^{14}\text{C}]\text{glucose}$.

Many investigations of the shunt are concerned with the percentage of glucose 6-phosphate going into the shunt and into glycolysis. The relative rates of reaction of glucose 6-phosphate dehydrogenase and phosphoglucose isomerase change continuously with time. Whereas the rate of glucose 6-phosphate dehydrogenase depends on both the rate of glucose 6-phosphate formation by hexokinase and the rate of NADP^+ regeneration, the rate of phosphoglucose isomerase depends only on the rate of glucose 6-phosphate formation. Thus, as NADP^+ regeneration by fatty acid synthesis falls during the 3 h incubation (regeneration by 'malic' enzyme does not decrease), the initial competitive advantage of glucose 6-phosphate dehydrogenase is lost and its rate decreases from 1.5 times that of phosphoglucose isomerase (at 20 min) to 0.5 (at 3 h).

Phosphoribose isomerase (EC 5.3.1.6), D-ribulose 5-phosphate 3-epimerase (EC 5.1.3.1), transketolase (EC 2.2.1.1) and transaldolase (EC 2.2.1.2)

When the model was first constructed, assays of these enzymes had not been performed in lactating rat mammary gland. Therefore rate constants for these reactions were derived solely on the basis of data from experiments using labelled compounds, which suggested that the enzymes of the pentose cycle act as a constant-proportion group, which is not rate-limiting. Subsequent measurements of enzyme activities of the pentose cycle by Baldwin & Milligan (1966) 'pentosephosphophosphatemetabolizing activity', Novello & McLean (1968) and Gumaa *et al.* (1971, 1973*a*) were in accord with this. It appears that in this tissue, unlike some others, all reactions of the shunt are going forward.

A number of assumptions were made in calculating rate constants for this group of reactions; the net fluxes are determined by the net flux through the 6-phosphogluconate dehydrogenase reaction. The activities of ribose 5-phosphate isomerase, ribulose 5-phosphate epimerase, transketolase and transaldolase are all high in this tissue. The steady-state concentrations of the intermediates are of the same order of magnitude as the equilibrium concentration of fructose 6-phosphate (from the phosphoglucose isomerase reaction).

Although there is experimental evidence in some tissues (Shaw & Stadie 1959; Hanson & Biorck, 1957; London, 1966; Landau & Sims, 1967) for compartmentation of metabolites (e.g. fructose 6-phosphate and glucose 6-phosphate), it has been assumed here that all pathways freely share common pools of metabolites, and this assumption has not caused difficulty. Because the K_m values for the transaldolase and transketolase reactions are similar in magnitude, their net fluxes were assumed equal, and in the direction:

This assumption is strengthened by the fact that transketolase takes part in two of the above three reactions, and transaldolase in one; Novello & McLean (1968) found the transketolase activity in lactating rat mammary gland to be twice that of transaldolase. These reactions produce the glycolytic intermediates fructose 6-phosphate and glyceraldehyde 3-phosphate, paralleling the glycolytic reactions and by-passing the slow phosphoglucose isomerase step.

Since xylulose 5-phosphate is used up in two of the above reactions, and ribose 5-phosphate in only one, the rate of conversion of ribulose 5-phosphate into xylulose 5-phosphate was set at twice that for its conversion into ribose 5-phosphate. Novello & McLean (1968) noted that in all tissues so far studied, ribulose 5-phosphate 3-epimerase activity (ribulose 5-phosphate $\rightarrow$ xylulose 5-phosphate) is considerably higher (about tenfold) than the ribose 5-phosphate isomerase activity (ribulose 5-phosphate $\rightarrow$ ribose 5-phosphate).

The transaldolase exchange reaction (Ljungdahl *et al.*, 1961) (which exchanges C-4, C-5 and C-6 of fructose 6-phosphate with glyceraldehyde 3-phosphate) was assumed to be insignificant in this study for the following reasons. The K_m of transaldolase for fructose 6-phosphate is twice that for sedoheptulose 7-phosphate. The contribution of recycling of the pentose-phosphate pathway to randomization of the radioactive label (and changes in $^{14}\text{CO}_2$ production from $[1-^{14}\text{C}]\text{glucose}$) is relatively small. This lack of recycling arises because the glycolytic-chain reactions are not very reversible. In our 'labelled' models, the amount of glucose 6-phosphate labelled differently from the substrate glucose was less than one-tenth of the concentration of glucose 6-phosphate labelled similarly. The transaldolase-exchange reaction will have a substantial effect only on ^{14}C distribution in hexose derivatives such as glycogen, which are not being considered here.

Phosphofructokinase (EC 2.7.1.11)

The complexity of the kinetics of this enzyme and the multiplicity of its control mechanisms (Garfinkel, 1965, 1966a; Lowry & Passoneau, 1966) make its role difficult to evaluate. In the present model, phosphofructokinase is considered not to be rate-limiting for the following reasons: the K_m values of this enzyme for fructose 6-phosphate and ATP are lower than the observed K_m values for most enzymes in lactating mammary gland [for partially purified phospho-

fructokinase from sheep brain the K_m for ATP = $1 \times 10^{-5}\text{M}$ and the K_m for fructose 6-phosphate = $4 \times 10^{-5}\text{M}$ (Lowry & Passoneau, 1966)]. The concentration of ATP found in slices of lactating rat mammary gland [$32.3 \mu\text{mol}/100\text{g}$ of tissue for the eleventh day of lactation (Wang & Greenbaum, 1960)] is in the concentration range shown by Lowry & Passoneau (1966) to be only moderately inhibitory.

Assuming this reaction to be non-rate-limiting helps to account for the high rate of production of $^{14}\text{CO}_2$ from $[3,4-^{14}\text{C}]\text{glucose}$ and of ^{14}C -labelled fatty acids from $[6-^{14}\text{C}]\text{glucose}$.

The concentration of metabolites preceding phosphofructokinase (e.g. glucose 6-phosphate, fructose 6-phosphate) did not increase with time (see Table 2).

Aldolase (EC 4.1.2.13)

This reaction was considered not to be rate-limiting, and its net flux was set equal to that of phosphofructokinase.

Triose phosphate isomerase (EC 5.3.1.1)

Since the amount of this enzyme is typically large, this reaction was assumed not to be rate-limiting. The net flux through this reaction was assumed to be towards formation of glyceraldehyde 3-phosphate because the turnover of this substance is more rapid than the formation of α -glycerophosphate and glycerol.

Glycerol formation

No measurement has been made of the rate of glycerol formation in this tissue, and this rate is not pertinent to our principal aim of studying interactions between the major pathways of glucose

catabolism. Glycerol formation was adjusted to hold glycerol near its starting concentration.

Glyceraldehyde 3-phosphate dehydrogenase (EC 1.2.1.12), *phosphoglycerokinase* (EC 2.7.2.3), *phosphoglyceromutase* (EC 2.7.5.3), *enolase* (EC 4.2.1.11) and *pyruvate kinase* (EC 2.7.1.40)

This sequence of reactions is assumed not to be rate-limiting and is represented by one lumped irreversible reaction:

To explain the high rate of production of $^{14}\text{CO}_2$ from [3,4- ^{14}C]glucose (arising in large part from pyruvate decarboxylation) and the high rate of ^{14}C -labelled fatty acid production from [6- ^{14}C]glucose, it was necessary to assume that all the glyceraldehyde 3-phosphate formed, from whatever source, goes through this reaction sequence. As noted above, most of the dihydroxyacetone 3-phosphate formed by aldolase is converted into glyceraldehyde 3-phosphate. Therefore the rate of this reaction sequence was set equal to the sum of the net rate of glyceraldehyde 3-phosphate production in the pentose cycle plus twice the rate of the aldolase reaction.

Pyruvate dehydrogenase (EC 1.2.2.2)

The rate of this reaction was chosen to give a close approximation to the rate of $^{14}\text{CO}_2$ production from [3,4- ^{14}C]glucose.

CO_2 fixation

Abraham *et al.* (1957) reported $^{14}\text{CO}_2$ production from [3,4- ^{14}C]glucose to be about two-thirds that from [1- ^{14}C]glucose, whereas Katz & Wals (1972) found that the $^{14}\text{CO}_2$ yield from [3,4- ^{14}C]glucose and from [3- ^{14}C]glucose exceeded that from [1- ^{14}C]glucose. The model could be made to fit either set of data by altering one assumption. To make the model agree with the findings of Abraham *et al.* (1957), which form the basis of this model, especially the formation of ^{14}C -labelled fatty acids and $^{14}\text{CO}_2$, it was necessary to have a significant amount of pyruvate by-pass the pyruvate dehydrogenase reaction by fixing CO_2 and entering the tricarboxylic acid cycle directly. The model can be fitted to the data of Katz & Wals (1972) either by increasing the activity of pyruvate dehydrogenase or by eliminating CO_2 fixation; as shown below, the first of these seems more correct.

When CO_2 fixation occurs via 'malic' enzyme, malate is formed and NADPH is oxidized to NADP^+ ; when it occurs via pyruvate carboxylase, oxaloacetate is formed. Both enzymes are present in lactating rat mammary gland (Gul & Dils, 1969).

In the model, CO_2 fixation by pyruvate was represented by the equation:

where CITOC represents the sum of all the C_4 compounds (and substances equilibrated with them) in the tricarboxylic acid cycle. The role of 'malic' enzyme in this process is described above.

Lactate dehydrogenase (EC 1.1.1.27)

Although the lactate concentration is high in the lactating rat mammary gland, there was little net flux toward either synthesis or breakdown at any time during the 3 h incubation.

Fat synthesis

Fat synthesis was represented by one reversible reaction:

(In this context, 'fatty acids' includes both free fatty acids and triglycerides.) The forward rate of this reaction was assumed to be large and limited primarily by the availability of acetyl-CoA, a function of both its rate of formation and its consumption by citrate synthase.

Abraham & Chaikoff (1959) present evidence that the oxidation of acetyl groups via the tricarboxylic acid cycle is saturated at a low acetate concentration (0.005 M), whereas the fatty acid-synthesizing system is not saturated at a higher acetate concentration (0.03 M). The rate constant for the forward direction towards fatty acid formation was therefore considerably higher than for the reverse direction at twice the rate of formation of ^{14}C -labelled fatty acid from [6- ^{14}C]glucose (since only 50% of the acetyl-CoA produced from [6- ^{14}C]glucose is labelled). The net flux of fatty acid formation could not be made this high without raising the rate of $^{14}\text{CO}_2$ formation from [3,4- ^{14}C]glucose considerably above Abraham & Chaikoff's (1959) experimentally determined values (which may be low, as noted above). This problem was surmounted by assuming the existence of a fairly large pool of endogenous fatty acids, which would

allow rapid ^{14}C -labelling of fatty acids with a much lower net rate of fatty acid synthesis. This assumption is supported by the finding of Wrenn *et al.* (1965) that the lowest value of fat observed in the lactating rat mammary gland was 12%. Other alternatives were tried, but this assumption always seemed necessary.

Ribose 5-phosphate + xylulose 5-phosphate $\rightarrow$ sedoheptulose 7-phosphate + glyceraldehyde 3-phosphate
 Erythrose 4-phosphate + xylulose 5-phosphate $\rightarrow$ fructose 6-phosphate + glyceraldehyde 3-phosphate

Tricarboxylic acid cycle

The tricarboxylic acid cycle is represented in the present model in a crude fashion, but by one that retains all the characteristics necessary for interpreting the labelling studies under consideration. The C_6 compounds are collectively represented by the symbol CIT2C, the C_5 compound α -oxoglutarate by CIT1C, and the C_4 compounds by the symbol CIT0C. Randomization is known to occur only at the level of the C_4 compounds. The active labelled forms would include corresponding ^{14}C -labelled amino acids, but as there are no data, accurate representation is impossible.

The net flux through the tricarboxylic acid cycle in the 'unlabelled' model was first set at twice the rate of $^{14}\text{CO}_2$ production from $[6\text{-}^{14}\text{C}]\text{glucose}$, then modified to fit the data.

Changes in flux throughout the incubation period were represented as secondary effects of changes in substrate concentration, particularly in acetyl-CoA and pyruvate (which directly affects CO_2 fixation), as a result of generalized slowing of glycolysis and the pentose cycle.

Lactose formation

Lactose formation shares two substrates with the glycolytic pathway, i.e. glucose and glucose 6-phosphate. Glucose has been shown to act as the galactosyl acceptor during lactose biosynthesis, and glucose 6-phosphate is an intermediate in the synthesis of UDP-galactose. Since the primary aim of this model was to determine the relation between glycolysis and the pentose cycle, lactose synthesis was represented by a simple unidirectional equation occurring at the glucose level. Although a more detailed representation is possible, its only effect of interest would be to alter the amount of available glucose 6-phosphate, which we already know is a controlling factor.

The rate of lactose production was set at approximately one-third of the CO_2 production by 6-phosphogluconate dehydrogenase, since Bartley *et al.* (1966) showed that when lactating rat mammary-gland slices were incubated with $[1\text{-}^{14}\text{C}]\text{glucose}$, the

^{14}C recovered in lactose was about one-third of that recovered in CO_2 .

Glyceraldehyde 3-phosphate

The only pentose-cycle reaction involved in substantial synthesis of glyceraldehyde 3-phosphate is transketolase:

At 20 min, the flux through these reactions is approximately 25% of the rate of formation of glyceraldehyde 3-phosphate from fructose 1,6-diphosphate by aldolase and triose phosphate isomerase. As the flux through the transketolase reactions decreases more rapidly than that of the aldolase, the percentage falls to about 16% at 60 min, and then to about 12% over the remainder of the 3 h period. Part of the glyceraldehyde 3-phosphate is taken up in the transaldolase reaction (sedoheptulose 7-phosphate + glyceraldehyde 3-phosphate $\rightarrow$ fructose 6-phosphate + erythrose 4-phosphate), the rest going to pyruvate; the rate of the first of these pathways is always at least an order of magnitude less than the second. Total utilization matches total production to two significant figures.

Fit to the Data

A single set of kinetic parameters has been developed (see 'unlabelled' model, Scheme 1 and Table 3) which, when substituted into the appropriately 'labelled' models, closely approximates the experimental values reported by Abraham *et al.* (1957) for ^{14}C recovery, as $^{14}\text{CO}_2$ and ^{14}C fatty acids, from $[1\text{-}^{14}\text{C}]\text{glucose}$ and $[6\text{-}^{14}\text{C}]\text{glucose}$ at the end of 1, 2 and 3 h, and from $[3,4\text{-}^{14}\text{C}]\text{glucose}$ at the end of 3 h (see Table 1). These same parameters, when used in the 'unlabelled' model, give a close approximation to the concentration of several metabolic intermediates measured by Dr. R. A. Frenkel (Table 2), under conditions identical with those of Abraham *et al.* (1957). The calculated values in most cases are within the range of experimental error of the measured ones, and agree within a factor of 2 in all cases. The calculated concentrations of substrates other than those measured for this study (Table 5) are often comparable with those measured by Gumaa *et al.* (1971) especially at zero time. The model is consistent with the enzyme measurements of Baldwin & Milligan (1966) and Gumaa *et al.* (1971, 1973a).

It should be noted that glucose 6-phosphate in the model shows a 'spike', to a maximum value of $0.26\text{ }\mu\text{mol}/0.5\text{ g}$, at 3 min, a time when no experiment

was done. Comparable glucose 6-phosphate 'spikes' have been seen elsewhere (Garfinkel & Hess, 1964).

Several features of both the data obtained by using radioactively labelled compounds and the models are significant; the rate of production of $^{14}\text{CO}_2$ from $[1-^{14}\text{C}]\text{glucose}$ decreases during the second and third hours (to about 50% of the rate for the first hour). The rate of production of $^{14}\text{CO}_2$ from $[6-^{14}\text{C}]\text{glucose}$ is highest during the second hour (about 1.5 times the average of the first and third hour). The low rate during the first hour is accounted for by assuming a build-up of tricarboxylic acid-cycle intermediates, which must occur somewhere after citric acid formation (as acetyl-CoA is known to be incorporated quite readily into the cycle). Segal *et al.* (1961) found a lag period preceding a linear increase of specific radioactivity of expired CO_2 after administering $[6-^{14}\text{C}]\text{glucose}$ to human subjects, but not after $[1-^{14}\text{C}]\text{glucose}$. The decreased rate during the third hour is due to a decreased availability of acetyl-CoA.

The rate of production of ^{14}C -labelled fatty acids from both $[1-^{14}\text{C}]\text{glucose}$ and $[6-^{14}\text{C}]\text{glucose}$ is lower during the second and third hours than during the first. (In the model, it is lower during the third hour than during the second.) This decrease reflects the decrease in available glucose 6-phosphate and NADP^+ . Rates of production of $^{14}\text{CO}_2$ from $[1-^{14}\text{C}]\text{glucose}$, $[6-^{14}\text{C}]\text{glucose}$ and $[3,4-^{14}\text{C}]\text{glucose}$ can be compared with the rates of those reactions in the 'unlabelled' model that are primarily producing the CO_2 , i.e. from $[1-^{14}\text{C}]\text{glucose}$, the 6-phosphogluconate dehydrogenase reaction, from $[6-^{14}\text{C}]\text{glucose}$,

the tricarboxylic acid-cycle reaction and from $[3,4-^{14}\text{C}]\text{glucose}$, the pyruvate dehydrogenase reaction. This comparison, shown in Table 4, illustrates the internal consistency of the model. The percentage of added ^{14}C recovered as $^{14}\text{CO}_2$ from $[1-^{14}\text{C}]\text{glucose}$ is somewhat higher than CO_2 production by 6-phosphogluconate in the 'unlabelled' model. This is due to the contribution of the tricarboxylic acid cycle toward the production of $^{14}\text{CO}_2$ from $[1-^{14}\text{C}]\text{glucose}$. Production of $^{14}\text{CO}_2$ from $[6-^{14}\text{C}]\text{glucose}$ is approx. 25% of the total amount of CO_2 produced by the tricarboxylic acid cycle after the first hour, since four carbon atoms enter the cycle from every molecule of $[6-^{14}\text{C}]\text{glucose}$. The percentage of added ^{14}C recovered as $^{14}\text{CO}_2$ from $[3,4-^{14}\text{C}]\text{glucose}$ is close to the amount of CO_2 produced by pyruvate dehydrogenase. This is because all the glyceraldehyde 3-phosphate (and hence pyruvate) produced by the glycolytic pathway, and at least 50% of that produced by the pentose cycle, is labelled in the C-1 position, which is removed as CO_2 by pyruvate dehydrogenase. Net fatty acid synthesis by the 'unlabelled' model (shown in Table 4) increases rapidly, then levels off. In the models for $[1-^{14}\text{C}]\text{glucose}$ and $[6-^{14}\text{C}]\text{glucose}$ the incorporation of ^{14}C into fatty acids shows a similar pattern.

The values obtained with the model for $[6-^{14}\text{C}]\text{glucose}$ are uniformly about 60% of those observed. This is due to the necessity of keeping the pyruvate dehydrogenase activity low enough to match the observed yields from $[3,4-^{14}\text{C}]\text{glucose}$. It is possible that the latter are actually too low, as Katz & Wals (1972) did obtain a significantly higher yield of labelled products from $[3,4-^{14}\text{C}]\text{glucose}$ (which could be reproduced by increasing pyruvate dehydrogenase activity, as noted above). [We have avoided mixing data from different experiments in this model, and the work of Katz & Wals (1972) could not be substituted for that of Abraham *et al.* (1957) as the starting point of the model because the former authors did not measure enough data points.]

There is no direct comparison between the intermediates in the tricarboxylic acid cycle of the model and of the tissue because the cycle is represented in a very rudimentary way in the model with the only goal being adequate representation of the data obtained by using labelled compounds. The 'intermediates' include the corresponding amino acids (aspartate, glutamate) with which they are interconverted by transamination. Even with this crude representation, some qualitative predictions of the model are borne out. The requirement of the model that citrate synthesis is reversible (in order to transmit sufficient ^{14}C to fatty acids via acetyl-CoA) has been effectively confirmed by Gumaa *et al.* (1973b); the neglect of NADPH production by isocitrate dehydrogenase was subsequently justified by the data of Gumaa *et al.* (1973a).

Table 4. Comparison of $^{14}\text{CO}_2$ recovery from $[1-^{14}\text{C}]\text{glucose}$, $[6-^{14}\text{C}]\text{glucose}$ and $[3,4-^{14}\text{C}]\text{glucose}$ with CO_2 production by those reactions in the 'unlabelled' model assumed to be the principal sources of the $^{14}\text{CO}_2$

	'Labelled' model Percentage of added ^{14}C recovered as $^{14}\text{CO}_2$	'Unlabelled' model CO_2 production ($\mu\text{mol}/0.5\text{ g}$ of tissue)
	$[1-^{14}\text{C}]\text{Glucose}$	6-Phosphogluconate dehydrogenase ($\times 2$)
1 h	9.8	7.4
2 h	15.4	9.8
3 h	19.6	11.4
	$[6-^{14}\text{C}]\text{Glucose}$	Tricarboxylic acid cycle ($\times 0.5$)
1 h	0.5	2.8
2 h	1.5	6.0
3 h	2.3	8.1
	$[3,4-^{14}\text{C}]\text{Glucose}$	Pyruvate dehydrogenase ($\times 1$)
1 h	6.4	8.9
2 h	11.9	12.0
3 h	15.7	13.6

Comparison of other models

The principal difference in assumptions between our model and those of Wood, Katz, and Landau (Wood *et al.*, 1963; Landau *et al.*, 1964; Katz *et al.*, 1966; Landau & Bartsch, 1966; Katz & Rognstad, 1967) is that ours does not require either metabolic or isotopic steady state. The percentage contribution of the pentose cycle in our model, obtained by using the definition of the pentose cycle of Katz & Wood (1963), is shown in Table 7. (This percentage may be taken at either 6-phosphogluconate dehydrogenase or glucose 6-phosphate dehydrogenase, with only slight differences in outcome.) There is a rapid decrease in the percentage contribution of the pentose cycle during the first hour, a much slower decrease during the second hour and a negligible decrease during the third hour. The percentage at the end of the first hour (24.0%) is similar to that calculated by Greenbaum & Darby (1964), who applied the formulae of Katz & Wood (1963) to their own data on lactating rat mammary glands and arrived at the value of 25%. An average of the percentages at the midpoint of the first, second, and third hours (43.4, 20.7, 16.4; mean = 26.8) gives a value close to that of Greenbaum & Darby (1964).

Since the 'labelled' models provide concentrations at specific times for each ^{14}C -labelled form of the metabolic intermediates, direct comparison is also possible between such models and the equations derived by Wood, Katz and Landau and co-workers (for references see above) for incorporation of ^{14}C into specific positions in triose phosphate derivatives or into hexose phosphate derivatives.

The model of Segal *et al.* (1961), although it applied to the overall contribution of the pentose cycle to metabolism *in vivo*, is also important. Although Segal *et al.* (1961) neglected recycling of the

hexose 6-phosphate formed by the pentose cycle, they made a significant contribution by treating the system in the non-steady state. Their finding of a lag period before production of $^{14}\text{CO}_2$ from $[6\text{-}^{14}\text{C}]\text{-glucose}$, but not from $[1\text{-}^{14}\text{C}]\text{-glucose}$, correlates well with our finding that CO_2 production by the tricarboxylic acid cycle increases after that by the pentose cycle has passed its peak.

Although not proposing a formal model, Novello & McLean (1968) present evidence that the enzymes of the pentose cycle in this tissue behave as a constant-proportion group of enzymes, and that the dehydrogenase reactions, rather than the transaldolase and transketolase reactions, are rate-limiting. Both of their conclusions are borne out by the present model.

Discussion

A number of biochemically important results emerge from this study. These include regulation of glucose 6-phosphate concentration by limiting glucose entry into the cell and inhibition of hexokinase, regulation of glucose 6-phosphate dehydrogenase by NADP^+ concentration, and fixation of pyruvate to oxaloacetate and malate.

This model suggests that glucose catabolism in the lactating rat mammary gland is controlled primarily by substrate and coenzyme concentration, rather than enzyme activities. The steady-state concentrations of the substrates in the models presented are much lower than their K_m values. A similar situation has been noted for perfused rat liver (Achs *et al.*, 1971).

Further, it has been possible to construct this model by assuming that each substance occurs in only one pool, even though there is evidence for some compartmentation of glycolytic intermediates (Achs

Table 5. Concentrations of substances as a function of time (in $\mu\text{mol}/0.5\text{g}$ wet wt. of tissue), calculated by using the 'unlabelled' model

Substance	Time (min)	...	0	20	40	60	120	180
Pyruvate			0.059	0.211	0.171	0.136	0.088	0.067
Lactate			6.40	6.48	6.53	6.56	6.56	6.50
6-Phosphogluconate			0.094	0.340	0.681	0.889	1.03	1.02
6-Phosphogluconolactone			0.094	0.650	0.523	0.411	0.296	0.264
C ₃ sugars			1.50	1.21	1.06	0.856	0.606	0.534
Erythrose 4-phosphate			0.004	0.009	0.009	0.008	0.006	0.005
Sedoheptulose 7-phosphate			0.060	0.054	0.048	0.040	0.031	0.029
Acetyl-CoA			0.100	0.066	0.037	0.021	0.007	0.004
Total CO_2			0	7.19	13.2	18.3	28.8	35.6
NADP^+			0.019	0.698	0.528	0.398	0.303	0.308
NADPH			3.80	3.12	3.29	3.42	3.52	3.51
Fatty acids			6.667	11.0	13.5	14.9	16.9	17.8
Lactose			0	0.393	0.780	1.14	2.06	2.77
Tricarboxylic acid-cycle intermediates (CIT0C+CIT1C+CIT2C)			0.414	1.27	3.22	5.62	13.3	20.7

Table 6. *Other significant fluxes in the model*Fluxes are expressed in $\mu\text{mol}/\text{min}$ per 0.5 g of tissue.

Time (min) ...	20	40	60	120	180
Hexokinase	0.1743	0.1518	0.1339	0.1097	0.0949
Glucose 6-phosphate dehydrogenase	0.1110	0.0800	0.0570	0.0360	0.0300
6-Phosphogluconate dehydrogenase	0.0990	0.0730	0.0550	0.0360	0.0320
Phosphoglucose isomerase	0.0630	0.0706	0.0762	0.0741	0.0647
Phosphofructokinase	0.1292	0.1271	0.1186	0.0999	0.0864
Transaldolase	0.0330	0.0282	0.0211	0.0128	0.0108
Pyruvate dehydrogenase	0.2110	0.1712	0.1364	0.0880	0.0669
Fatty acid synthesis	0.1592	0.0947	0.0562	0.0196	0.0100
First tricarboxylic acid-cycle decarboxylation	0.0469	0.0756	0.0808	0.0692	0.0574
CO ₂ fixation by pyruvate	0.0758	0.1133	0.1248	0.1269	0.1192
NADPH $\rightarrow$ NADP ⁺	0.0468	0.0494	0.0513	0.0528	0.0527

Table 7. *Percentage of glucose utilized by the pentose cycle*

This (%PC) was calculated at both glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase from the formula:

$$\%PC = \frac{1}{3} \left[\frac{\text{Flux (glucose 6-phosphate dehydrogenase)}}{\text{Flux (phosphoglucose isomerase)}} \right] \times 100$$

Time (min)	Flux (phosphoglucose isomerase)	Flux (6-phosphogluconate dehydrogenase)	%PC	Flux (glucose 6-phosphate dehydrogenase)	%PC
20	0.0630	0.099	52.4	0.111	58.7
40	0.0706	0.073	34.5	0.080	37.8
60	0.0762	0.055	24.0	0.057	24.9
80	0.0774	0.45	19.4	0.045	19.4
100	0.0763	0.040	17.5	0.039	17.0
120	0.0741	0.036	16.2	0.036	16.2
180	0.0647	0.032	16.5	0.030	15.4

et al., 1971). In part, this may be due to the long time-scale of the experiments; slowness of mixing would probably not be detected in a 3h experiment. Although this implies complete equilibration of glyceraldehyde 3-phosphate from any source with glyceraldehyde 3-phosphate from any other, it does not imply complete equilibration of glyceraldehyde 3-phosphate with dihydroxyacetone phosphate (the triose phosphate isomerase reaction in this model is not a really rapid exchange).

The relative activities of phosphoglucose isomerase and phosphofructokinase in lactating rat mammary gland appear quite different from those in most other tissues. Ordinarily, phosphoglucose isomerase is present in considerable excess, and keeps glucose 6-phosphate and fructose 6-phosphate at equilibrium, and phosphofructokinase is the glycolytic enzyme present in smallest amount, and the one which is considered to be controlling. Phosphofructokinase is inhibited by citrate, a tricarboxylic acid-cycle intermediate, and phosphoglucose isomerase by 6-phosphogluconate, a shunt intermediate, so that in most

tissues glycolysis is regulated by the tricarboxylic acid cycle rather than the shunt. In mammary gland the situation appears reversed: phosphofructokinase is not particularly limiting (its flux equals the net production of fructose 6-phosphate) and phosphoglucose isomerase is closer to being limiting than usual; it is obviously not present in the usual great excess (Table 7). This control point for glycolysis is regulated by the shunt and is sensitive to hormonal control. The shunt allows a significant amount of glucose 6-phosphate to bypass the relatively slow phosphoglucose isomerase step. This effect decreases with time, the glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase fluxes falling as a result of the decreased availability of NADP⁺, whereas the phosphoglucose isomerase flux increases relatively. The other glycolytic reactions appear not to be rate-limiting and decrease with time as a reflection of the rates of the hexose-metabolizing enzymes (hexokinase, phosphoglucose isomerase and glucose 6-phosphate dehydrogenase).

This situation may benefit mammary gland, which

actively synthesizes fatty acid, in making a large amount of NADPH (generated by the dehydrogenase reactions of the shunt) available for such synthesis.

An important feature of the model which owes its presence to consideration of both ^{14}C kinetics and concentrations of unlabelled metabolites is the effective control of glucose 6-phosphate by NADP^+ via the glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase reactions, in accordance with the views of McLean (1960). This control manifests itself by a considerable variation with time (Tables 6 and 7) of the fluxes through the shunt, many of which change by a factor of 3 or more. (These reactions are always going forward, and are thoroughly interlinked.) As a result the proportion of glucose going through the pentose cycle varies with time and does not reach a steady-state value for about 2 h (Table 7).

As the model of Wood *et al.* (1963) requires steady state of both labelled and unlabelled intermediates, the question of the steady-state behaviour of this tissue requires some attention. The present model is in a near-steady state with regard to glycolytic material (Table 2) but not with regard to the pentose cycle (Table 7). An earlier version of this model did have a constant percentage of material going through the pentose cycle, but at the expense of having the calculated concentrations of Table 2 being both considerably further from a steady state and a considerably worse fit to the data. (Most of the calculated concentrations at 3 h were low by a factor of at least two, often more.) In particular, the varying percentage flow through the shunt was necessary to obtain good agreement with R. Frenkel's (personal communication) data for glucose 6-phosphate concentration. This suggests that the closer the glycolytic pathway comes to a steady state, the further from it the pentose cycle will be, and conversely.

It was possible to check this apparent pattern by intervening to remove the 'near' from the near-steady-state character of the glycolytic intermediates in Table 2 (this type of manipulation is possible only with a model). Since the model has reached the near-steady state at 20 min, the calculation was changed at that time to keep the concentrations of substances tabulated in Table 2 constant at their 20-min values, and the calculation was then repeated up to a time of 180 min. There was little change in the behaviour of the system, but it was all in the direction of taking even longer for the pentose cycle to reach a steady state, so that the 1 h, 2 h, and 3 h percentages going through the pentose cycle measured at glucose 6-phosphate dehydrogenase were now 30.3, 21.8 and 17.6% (i.e. nearly 3 h are now required to reach the steady state). This strengthens the suggestion stated above.

Most of the non-steady-state behaviour of the pentose cycle is due to the behaviour of the NADP^+ -

NADPH couple, which becomes rapidly oxidized initially and then slowly reduced again (similarly to what is shown in Table 5). It is possible that the model understates the effect of this behaviour in that we have not included the inhibition of glucose 6-phosphate dehydrogenase by NADPH observed by Glaser & Brown (1955) for the yeast enzyme, which would amplify the effects of the changes in NADP^+ . It is also possible that this phenomenon might be mitigated by appropriate pre-treatment of the slices (such as a priming dose), but we have not been able to analyse this situation.

The possibility that this result is a preparation-dependent artifact is diminished by the fact that the model can be reasonably made to fit the data of Katz & Wals (1972) with only one adjustment (to pyruvate dehydrogenase, an enzyme with very complex controls), although the preparation of Katz & Wals (1972) is quite different from that of Abraham *et al.* (1957) and might have been expected to lead to more differences.

As a very considerable effort to build a model which is in steady state with respect to everything and which matches all the available data has been unsuccessful, the possibility must be considered that it is impossible to do so for lactating rat mammary-gland slices. Such a conclusion would be strengthened by availability of a more definitive set of data and perhaps a more sophisticated model.

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