Estimations of Pathway Contributions to Glucose Metabolism and the Transaldolase Reactions*

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SUMMARY

A model for the estimation of pathway contributions has been extended to include the effect of the transaldolase reactions on the randomization of 14C of specifically labeled glucose. With glucose-2-14C as substrate, the contribution of the pentose cycle to glucose metabolism and the extent of isomerization of fructose 6-phosphate to glucose 6-phosphate in a tissue represented by the model can be estimated solely from the distribution of 14C in the 6 carbon atoms of glucose 6-phosphate or a derivative. Data for adipose, adrenal, and thyroid tissue support the adequacy of this approach. In these tissues, the transaldolase exchange reaction, as well as the reaction of the pentose cycle catalyzed by transaldolase, appears to introduce 14C from glucose-2-14C into carbon atoms 4, 5, and 6 of hexose-6-P. The maximum rate of the transaldolase exchange reaction can be estimated. The transaldolase reactions do not affect estimates of pentose cycle contributions with glucose-1-14C and -6-14C when triose-P derivatives are employed, but do alter the relative specific activities in carbon atoms 1 to 6 of hexose-6-P derivatives.

In a previous paper (1), methods were presented for the estimation of the contribution to glucose metabolism of biochemical pathways occurring in tissues. A model containing pools of intermediates and rates of reactions was assumed to describe the pathways as they function within a tissue. By determining the distribution of 14C in carbon atoms 1, 2, and 3 of glucose 6-phosphate and fructose 6-phosphate in a tissue presented with glucose-2-14C, and containing the pathways as assumed in the model, the relative rates of the reactions and thus the relative contributions of the pathways could be estimated. In making these estimations, no assumption of the rate of isotopic equilibration of fructose-6-P with glucose-6-P, i.e. the extent of recycling, was required. Solution of the model also proved possible with the use of glucose-1-14C and -6-14C as substrates. However, when yields of carbon atoms 1 and 6 in CO2 and triose-P derivatives were the determinants, the rate of isotopic equilibration of triose phosphates had to be considered (2).

No provision was made in the model for reactions catalyzed by transaldolase. The first of these occurs in the pentose cycle (Reaction 3).

3 Glucose-6-P → 3 CO2 + 3 pentose 5-P
2 Pentose-5-P ← sedoheptulose-7-P + glyceraldehyde-3-P (2)

transaldolase
Glyceraldehyde-3-P + sedoheptulose-7-P → fructose-6-P + erythrose-4-P (3)

Erythrose-4-P + pentose-5-P → fructose-6-P + glyceraldehyde-3-P (4)

2 Fructose-6-P → 2 glucose-6-P (5)

Reaction 2 of the cycle results in the formation of 1 molecule of glyceraldehyde-3-P. This glyceraldehyde-3-P enters the pool of glyceraldehyde-3-P in a system represented by the model. A molecule of glyceraldehyde-3-P then re-enters the cycle via Reaction 3 to provide carbon atoms 4, 5, and 6 of fructose-6-P (this will be referred to for convenience as the "pickup" reaction of the cycle).

The transaldolase exchange reaction (3) also introduces the carbon atoms of glyceraldehyde-3-P into carbon atoms 4, 5, and 6 of fructose-6-P.

Glyceraldehyde-3-P + fructose-6-P → fructose-6-P + glyceraldehyde-3-P (6)

The effect of these reactions on estimations of the contribution of pathways to glucose metabolism and methods for their estimation are now to be considered.

MODEL, FORMULATIONS, AND APPLICATIONS

The model of Fig. 1 is similar to that previously developed (1, 2) except for two additions. These are provisions for (a)
the entrance of glyceraldehyde-3-P formed via the pentose cycle into the glyceraldehyde-3-P pool and its return to the cycle, the pickup reaction, at the rate \( \frac{1}{3} V_1 \), and (b) the exchange of glyceraldehyde-3-P with fructose-6-P at the rate \( V_7 \). 

The consequence of these reactions when glucose-2 \(^{14}\)C is presented to the system is first considered. As previously discussed (1), the fructose-6-P formed via the pentose cycle will contain \(^{14}\)C in carbon atoms 1, 2, and 3. If there is no recycling, glucose-6-P will contain activity only in carbon 2. If recycling is complete, the specific activities of carbon atoms 1, 2, and 3 of glucose-6-P will be the same as those of fructose-6-P. If recycling is incomplete, there will be more \(^{14}\)C in carbon 2 relative to carbon atoms 1 and 3 in glucose 6-P than in fructose 6-P. Conversion of fructose-6-P to fructose-1,6-di-P at rate \( V_2 \) is assumed to be irreversible, so that net flow of carbon to dihydroxyacetone-P and glyceraldehyde-3-P is for each at the rate \( \frac{1}{3} V_1 \). The \(^{14}\)C in the carbon atoms of the dihydroxyacetone-P reflects the relative activities in carbon atoms 1, 2, and 3 of the fructose-6-P.

\[
\begin{array}{cccccccc}
1 & 2 & 3 & 4 & 5 & 6 \\
C & C & C & C & C & C & P \\
\end{array}
\]

Fruuctose-6-P-1,2,3-\(^{14}\)C

\[
\begin{array}{cccc}
V_2 \\
\downarrow \\
Fruuctose-1,6-di-P-1,2,3-\(^{14}\)C & V_1 \\
\end{array}
\]

Dihydroxyacetone-3-P-\(^{14}\)C Glyceraldehyde-3-P

Isotopic equilibration via triose-P isomerase at rate \( V_7 \) introduces \(^{14}\)C into glyceraldehyde-3-P. Via the pickup and exchange reactions (Equations 8 and 9, respectively), \(^{14}\)C would then appear in carbon atoms 4, 5, and 6 of fructose-6-P. To complete the cycle the fructose-6-P is isomerized to glyceraldehyde-6-P (Reaction 5). Therefore, carbon atoms 6, 5, and 4 of the fructose-6-P reflect the relative distribution of \(^{14}\)C that was in carbon atoms 1, 2, and 3 of the fructose-6-P cleaved to dihydroxyacetone-P and glyceraldehyde-3-P (Reaction 7).

\[
\begin{array}{cccccc}
2C & 3C & 3C \\
\rightarrow \\
4C & 4C & 5C \\
6CP & 6CP & 6CP \\
\end{array}
\]

Glyceraldehyde-3-P-\(^{14}\)C Sedoheptulose-7-P

\[
\begin{array}{cccccc}
1C & 2C & 3C \\
4C & 1 & 4C \\
5C & 5C & 6CP \\
6CP & 6CP & 6CP \\
\end{array}
\]

Fructose-6-P Glyceraldehyde-3-P-\(^{14}\)C

Thus, the degradation of glucose-6-P or a derivative of glucose-6-P, as glycogen, yields (a) from the distribution of \(^{14}\)C in carbon atoms 1, 2, and 3 of glucose-6-P, and (b) from the distribution in carbon atoms 6, 5, and 4 that in carbon atoms 1, 2, and 3 of fructose-6-P. Designating \( x_1, x_2, \ldots \), as the specific activities of the carbon atoms of glucose-6-P and \( y_1, y_2, \ldots \), as the specific activities of the carbon atoms of fructose-6-P (1): \( y_1/y_2 = y_4/y_5 = x_6/x_5 \).

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**Fig. 1.** Model of glucose metabolism. Glucose is metabolized to glucose-6-P at the rate \( V_0 \). The glucose-6-P is converted to fructose-6-P at the rate \( V_7 \) and the reverse reaction occurs at the rate \( V_{-7} \). The rate of entrance of glucose-6-P into the pentose cycle is \( V_1 \). One-third of this (\( \frac{1}{3} V_1 \)) is metabolized to \( CO_2 \) (\( \frac{1}{3} V_1 \)) and glyceraldehyde-3-P (\( \frac{1}{3} V_1 \)). One-sixth (\( \frac{1}{6} V_1 \)) is converted to glyceraldehyde-3-P and then to fructose-6-P with the remaining carbon atoms that enter the pentose cycle (\( \frac{1}{6} V_1 \)) forming fructose-6-P at the rate \( \frac{1}{6} V_1 \). Glyceraldehyde-3-P exchanges with the carbon atoms of fructose-6-P at the rate \( V_7 \). Metabolism via the Embden-Meyerhof pathway proceeds irreversibly at the rate \( V_7 \) to form dihydroxyacetone-P and glyceraldehyde-3-P. These equilibrate at the rates \( V_7 \) and \( V_{-7} \) in the directions indicated. Conversion to glycerol proceeds at the rate \( V_{7} \) and to lactate and the products of the Krebs cycle at the rate \( V_{11} \). Glucose-6-P is also converted via pathways not forming triose phosphate, as in glycojen formation at the rate \( V_{21} \). The fraction of glucose metabolized via the pentose cycle is \( \frac{1}{3} V_1/V_0 \) via the Embden-Meyerhof pathway is \( V_7/V_0 \) and via non-triose-P pathways, \( V_{11}/V_0 \). \( E_{-A} \) is defined as the rate of conversion of fructose-6-P to glucose-6-P as a fraction of total glucose utilization, \( V_{-A}/V_0 \). \( E_{\pi} \) is defined as the rate of exchange of glyceraldehyde-3-P with fructose-6-P as a fraction of total glucose utilization, \( V_{\pi}/V_0 \).
and $x_1/x_6 = y_1/y_6 = x_3/x_5$. If recycling is incomplete, $x_2/x_5 > y_1/y_6$. The mathematical derivation of these relationships is presented under “Appendix.”

Estimation of Pentose Cycle Contribution and Rate of Hexose-6-P Isomerization with Glucose-2-14C—Equations previously presented (1) remain valid when using glucose-2-14C in the estimation of the fraction of total glucose metabolized via the pentose cycle, $PC$, and the rate of isomerization of fructose-6-P to glucose-6-P, $E_{-h} = V_{t}/V_{o}$. However, if the determination of the distribution of 14C in derivatives of both glucose-6-P and fructose-6-P is unnecessary. A single complete degradation of a glucose-6-P derivative is all that is required.

Example 1: The distribution of 14C in the glucose unit of glycogen on incubation of rat epididymal adipose tissue with glucose-2-14C in the presence of insulin was found to be (relative to carbon 2 as 100) 30.7, 100, 17.9, 3.5, 14.8, and 5.3 (Table II of Reference 4). The equations applicable, when a non-triose-P pathway contribution is assumed to be absent, are (1)

$$PC = \frac{(2 + x_1/x_2)y_1/y_6}{(6 - 5x_1/x_2)y_1/y_6 + (2 - x_1/x_2)(2 - 3x_1/x_2)}$$

$$E_{-h} = \frac{(2 - x_1/x_2 + 2y_1/y_6)z_2/z_8}{(y_1/y_6 - z_1/z_8)(2 - z_1/z_8)}$$

Substituting 30.7/100 for $x_1/x_2$ and 5.3/14.8 for $y_1/y_6$, since $y_1/y_6 = x_2/x_3$, $PC = 0.24$ and $E_{-h} = 8.5$. Therefore, 94% of the over-all glucose metabolized is via the pentose cycle and the rate of isomerization of fructose-6-P to glucose-6-P ($V_{t}/V_{o}$) is 8.5 times the rate of glucose utilization ($V_{o}$).

Relative Rate of Exchange Reaction—As is evident from Equations 1 through 5, for every 3 molecules of glucose-6-P entering the pentose cycle there is a net formation of 3 molecules of CO$_2$, 1 molecule of glyceraldehyde-3-P, and 2 molecules of fructose-6-P which are isomerized to glucose-6-P. Another molecule of glyceraldehyde-3-P is formed, but it is picked up as carbon atoms 4, 5, and 6 of fructose-6-P. The extent of this pickup ($V_{t}/V_{o}$) is determined by $PC$. Within the confines of the model, any other incorporation into carbon atoms 4, 5, and 6 must occur via the exchange reaction at the rate $V_{t}$.

Expressions for the rate of the transaldolase exchange reaction relative to glucose utilization, $V_{t}/V_{o}$, designated by $E_{xt}$, may be obtained in terms of the relative specific activities of carbon atoms 4, 5, and 6 relative to carbon atoms 1, 2, and 3 of glucose-6-P and fructose-6-P, when glucose-2-14C is presented to a system represented by the model. An explicit expression for $x_1/x_2$ in terms of $PC$, NTP, $E_{-h}$, and $V_{t}/V_{o}$ is given in Equation 7' of “Appendix.” The relationships $x_1/x_2 = x_3/x_5 = y_1/y_6 = x_2/x_3$ hold. NTP denotes the fraction of glucose metabolized via non-triose-P-forming pathways (1). The quantity $V_{t}/V_{o}$ is the ratio of the rate of conversion of dihydroxyacetone-P to glycerol to the rate of its conversion to glyceraldehyde-3-P. The drainage of the carbon atoms of dihydroxyacetone-P at the rate $V_{t}$ would be expected in most tissues to be very small compared to the quantity isomerized to glyceraldehyde-3-P and then converted to lactate, CO$_2$, via the Krebs cycle, fatty acids, etc. Even in adipose tissue where triglyceride glycerol formation is marked, the rate of such synthesis under most conditions is only a small fraction of the rate $V_{t}$ (2, 5, 6). When this ratio and the non-triose-P pathway contributions are negligible (NTP = 0), Equation 7' reduces and solving for $E_{xt}$

$$E_{xt} = 1/2 \left[ \frac{2PC}{1 + 1/E_{-h}} + 1 - 2PC \right] \left[ \frac{1 - PC}{2 - PC} \right] \frac{x_1}{x_6} - PC$$

If $V_{t}/V_{o}$ cannot be assumed to be negligible, Equation 7' of the “Appendix” may be solved for $E_{xt}$. A non-triose-P pathway such as lactate or fatty acids when the substrates are glucose-1-14C and -6-14C.

In Fig. 2, $E_{xt}$ for values for 0 to $\infty$ is graphed as a function of $x_1/x_2$ and PC with Equation 12 where the equilibration of the hexose 6-phosphates is assumed to be complete ($E_{-h} = \infty$). When $E_{xt}$ is 0, incorporation into carbon 6 occurs only via the pickup reaction, and the ratio $x_1/x_2$ is the maximum value that can be observed for a given PC. $E_{xt}$ cannot be measured accurately for values above 5, since $x_1/x_2$ changes little in the range from $E_{xt} = 5$ to $\infty$ for a given PC. When $E_{xt} = \infty$, the ratios are the minimum which can be observed if incorporation into carbon 6 is solely via the pickup and exchange reactions. Ratios beyond these limits indicate that either the assumptions for the plot or model are inadequate or the data employed are incorrect.

![Fig. 2. Ratio of incorporation of 14C of glucose-2-14C into carbon 1 compared to carbon 6 of glucose-6-P ($x_1/x_6$) as a function of pentose cycle (PC) and the rate of the transaldolase exchange reaction ($E_{xt}$). Non-triose-P pathways are assumed absent (NTP = 0) and isotopic equilibration of the hexose 6-phosphates and triose phosphates complete. PC is varied from 0 to 1.0 for values of $E_{xt}$ from 0 to $\infty$.](http://www.jbc.org/)
Example 2: In Example 1, $PC = 0.24$, $E_{-h} = 8.5$, and $x_1/x_6 = 30.7/5.3 = 5.8$. As evidenced by balance studies, non-triose-P pathways appear to make only a small contribution to total glucose utilization in adipose tissue. Thus, in the presence of insulin perhaps 3% of utilized glucose is converted to glycogen (6). If this is the major non-triose P pathway in the tissue, NTP may be taken as 0.03. The ratio $V_{gl}/V_0$ as already discussed is also usually small. In one experiment in which adipose tissue was incubated with insulin, it equaled 2.2/185 = 0.01 (Table 7c of Reference 6). Substituting these values into Equation 7, $E_{XT} = 0.20$. Therefore, the rate of exchange of the carbon atoms of glyceraldehyde-3-P with fructose-6-P is estimated to be 0.9 the rate of utilization of the carbon atoms of glucose. Since $V_1/V_6 = 3 PC = 0.72$, the relative rate of the pickup reaction, $\frac{V_1}{V_6}$ is about one-half the rate of the exchange reaction.

If recycling is assumed to be complete, $E_{-h} = \infty$, and $V_{gl}/V_t$ and NTP are assumed to be 0 from Equation 12 or as approximated from Fig. 2, $E_{XT} = 0.21$. Unless there is little recycling or non-triose-P pathway contributions are significant, or $V_{gl}/V_t$ is large, Equation 12 with $E_{-h} \rightarrow \infty$ then becomes an excellent approximation. If the exchange reaction had been absent ($E_{XT} = 0$) for a pentose cycle of 24%, the observed ratio of $x_1/x_6$ from Fig. 2 would have been expected to be 12.

Estimation of Pathway Contributions with Use of Glucose-1-14C and -6-14C—in the absence of a non-triose-P pathway contribution, the introduction of the transaldolase reactions, with glucose 1-14C and -6-14C as substrates, will not alter the specific activities of dihydroxyacetone-P and glyceraldehyde-P. This results from the fact that in the absence of a non-triose-P pathway there is no outflow of 14C except to CO2 and triose-P. Since there is no mechanism by which carbon 6 of glucose can be randomized to carbon 1 of glucose-6-14C, no 14C of glucose-6-14C can appear in CO2 formed via the pentose cycle. All of carbon 1 of glucose which is converted to triose-P and then becomes carbon 6 of fructose-6-P via the transaldolase reactions must reappear as triose-P, since carbon 6 of fructose-6-P must be metabolized to triose-P. The equations expressing these relationships are presented under “Appendix.”

However, the transaldolase reactions do alter the specific activity of carbon 6 of the hexose 6-phosphates.1 The expressions derived for a model not encompassing these reactions therefore require modification (Tables I and II of Reference 1).2 The resulting expressions are complex and of very limited value, but for completeness are presented in “Appendix.” The effect of the introduction of only the pickup reaction into the previous model (1) ($V_0 = 0$ in the model of Fig. 1), on the ratio of incorporation of 14C from glucose-1-14C to glucose-6-14C into carbon atoms 1 and 6 of glucose-6-P ($x_1/x_6$), is depicted in Fig. 3. A non-triose-P pathway contribution is assumed to be absent, and isotopic equilibration of the triose phosphates is assumed to be complete. When $E_{-h}$ is greater than 1, small changes in $x_1/x_6$ result in large changes in PC. In addition, when using ratios from incubations with glucose-1-14C and -6-14C, in contrast to glucose-2-14C, a value for $E_{-h}$ must be assumed. Further, estimates of PC from $x_1/x_6$ will be incorrect unless an adjustment can be made for the incorporation of 14C into carbon 6 of glucose-6-P via the exchange reaction. The curves in Fig. 3 are only applicable when glucose-1-14C and -6-14C are presented separately to the system and the specific activity in carbon atoms 1 and 6 of glucose-6-P, respectively, is determined.

Experimentally, it is convenient to incubate with glucose-1-14C and -6-14C in a single flask and determine the relative 14C activity in all of the carbon atoms of glycogen was measured rather than only into carbon 1. Since, via the transaldolase reaction, 14C of glucose-1-14C is introduced into carbon 6, the experimental ratios would be expected to be greater than the theoretical.

The limitations in the use of glucose-1-14C and -6-14C may be

1 Flatt and Bell (7) compared the calculated ratio of incorporation of 14C from glucose-1-14C and -6-14C into glycogen of adipose tissue, when recycling is present, with that experimentally observed. Agreement was not good. Higher experimental than theoretical values were observed. However, the formulas for recycling employed are for the ratio of 14C from carbon 1 of glucose into carbon 1 of the glucose unit of glycogen relative to the incorporation from carbon 6 of glucose into carbon 6 of the unit. In the experiments, incorporation of 14C from glucose-1-14C into all of the carbon atoms of glycogen was measured rather than only into carbon 1. Since, via the transaldolase reaction, 14C of glucose-1-14C is introduced into carbon 6, the experimental ratios would be expected to be greater than the theoretical.

2 J. P. Flatt (personal communication) has considered the effect of the pickup reaction, but not the exchange reaction, when glucose-1-14C and -6-14C are substrates and isotopic equilibrations are assumed complete. The equations he derived are equivalent to those presented here for that circumstance.
shown with the use of data from a previous paper. An incuba-
tion of a mixture of glucose-1-14C and -6-14C was performed with
adipose tissue in the presence of insulin (Table VI of Reference 
4). The ratio of the specific activity in carbon 1 relative to
carbon 6 of the glucose unit of glycogen was 0.744. If \( E_{1,6} = x \)
assumed to be \( x \) and the pickup reaction is neglected, from Fig.
3, PC = 0.17. If the pickup reaction is considered to be the only
mechanism by which \( ^14 \)C from glyceraldehyde-3-P can be in-
corporated into carbon 6 of glucose-6-P, PC can be estimated
from Fig. 3 to be between 0.17 and 0.23. Since the extent of
incorporation via the transaldolase exchange reaction cannot be
determined from the data, the error in the estimate of pentose
cycle occasioned by this reaction

The incorporation of \( ^14 \)C of glucose-1, 6-14C and glucose-2-14C
into glycogen served as one test for the validity of the previous
model, since for that model the relationship \( x_1/x_6 = 1 - x_3/x_4 \)
should hold (Equation 30 of Reference 1). In spite of the fact
that the previous model did not encompass the transaldolase
reactions, this relationship experimentally appeared to hold.
This is presumably a reflection of the small changes in \( x_1/x_6 \)
produced by inclusion of the transaldolase reactions.

DISCUSSION

Validity of Model—When glucose-2-14C is incubated with
various tissues, the glucose unit of glycogen is found to contain
14C in carbon atoms 4, 5, and 6 (4, 5, 8, 9). The reactions produc-
ing this incorporation were not included in previous models. It
was recognized that the reactions would not change \( ^14 \)C ratios of
carbon atoms 1, 2, and 3 of glucose-6-P and fructose-6-P which
were the determinants employed in the estimation of the pentose
cycle pathway contribution and extent of recycling (1, 10).
As shown in the present model, the distributions in carbon atoms
4, 5, and 6 of glucose-6-P or its derivative will mirror the dis-
tributions in carbon atoms 1, 2, and 3 of fructose-6-P. The ratios in
glycerol and glucose of \( ^14 \)C are also in accord with the model, for experiments
that have been reported, in which fructose-2-14C and mannose-2-
14C were substrates (Table V of Reference 4) and for experi-
ments with glucose-2-14C in the presence of growth hormone (4).
As shown in Table I, the ratio \( x_1/x_6 \) is in agreement with the
relationships predicted for the model when isotopic equilibration
of the hexose 6-phosphates is incomplete, since it is greater
than \( x_1/x_3 \) and \( x_2/x_4 \). In accord with the model, the ratios \( x_1/x_3 \)
and \( x_2/x_4 \) are similar. Distributions of \( ^14 \)C in the glucose from
glycogen isolated on incubation of glucose-2-14C with adipose
tissue in the presence of growth hormone (5) and with adrenal
and thyroid slices (8, 9) also fulfill these relationships. However,
the incorporation of activity in carbon atoms 6, 5, and 4 relative
to carbon atoms 1, 2, and 3 is greater than can be attributed
solely to the transaldolase pickup reaction of the pentose cycle.
This increased incorporation may be explained by the presence
of the exchange reaction in all three tissues.

\[ \text{Table I} \]

\begin{tabular}{|c|c|c|c|c|c|c|c|}
\hline
\textbf{Condition} & \textbf{Glycerol} & & & \textbf{Glucose from glycogen} \\
& \textbf{C-1:C-2} & \textbf{C-1:C-3} & & \textbf{C-1:C-2} & \textbf{C-1:C-3} & \textbf{C-4:C-5} & \textbf{C-6:C-5} & \textbf{C-1:C-6} & \textbf{C-2:C-5} & \textbf{C-3:C-4} \\
\hline
\text{No insulin} & & & & & & & & & & \\
\text{Experiment 3} & 0.299 & 0.104 & 0.199 & 0.119 & 0.151 & 0.216 & 0.03 & 7.19 & 5.67 \\
\text{Experiment 4} & 0.161 & 0.125 & 0.130 & 0.01 & 0.142 & 0.209 & 4.64 & 7.46 & 4.79 \\
\text{Experiment 8} & 0.229 & 0.152 & 0.184 & 0.179 & 0.135 & 0.197 & 5.26 & 5.62 & 7.46 \\
\text{Experiment 10} & 0.163 & 0.100 & 0.147 & 0.090 & 0.187 & 0.172 & 4.94 & 6.94 &  \\
\text{Mean} & 0.203 & 0.135 & 0.165 & 0.130 & 0.130 & 0.202 & 5.49 & 6.80 & 5.97 \\
\hline
\text{Insulin} & & & & & & & & & & \\
\text{Experiment 3} & 0.351 & 0.231 & 0.325 & 0.212 & 0.279 & 0.379 & 6.13 & 7.14 & 5.44 \\
\text{Experiment 5} & 0.371 & 0.221 & 0.293 & 0.174 & 0.226 & 0.359 & 5.70 & 7.04 & 5.44 \\
\text{Experiment 6} & 0.204 & 0.182 & 0.311 & 0.142 & 0.285 & 0.424 & 5.55 & 7.58 & 4.76 \\
\text{Experiment 10} & 0.235 & 0.122 & 0.294 & 0.187 & 0.230 & 0.356 & 3.72 & 4.50 & 3.67 \\
\text{Mean} & 0.305 & 0.189 & 0.305 & 0.184 & 0.248 & 0.380 & 5.28 & 6.57 & 4.83 \\
\hline
\end{tabular}

\(^a\) Calculated from the data of Tables II and III of Landau and Katz (4).

\(^b\) The C-3:C-2 ratio in glycerol is much lower than the C-6:C-5 ratio in glucose, but the C-3:C-2 ratio in glycerol is less than the
C-1:C-2 ratio in glucose.
The presence of growth hormone (5). Values for adrenal and thyroid tissue in the absence of added hormone and adipose tissue were based on the assumption of complete recycling, as evidenced by the failure of \(^{14}C\) of glucose-6-\(^{14}C\) to be randomized during its incorporation into glycogen (References 4 and 5 and Footnote 3). If reversal occurred, \(^{14}C\) from glucose-6-\(^{14}C\) should have been found in carbon 1 of the glucose unit of glycogen. In adipose tissue, negligible activity of fructose 1,6-P carbon atoms into carbon atoms 1, 2, and 3 predicted for the model with glucose-2-\(^{14}C\) as substrate. These are recorded in Table II. The pentose cycle contribution to glucose metabolism made in these tissues were based on the assumption of complete recycling, i.e., from the ratio of activity in C-1:C-2 with the use of the expression \(x_1/x_2 = 2/\text{PC} + 1\) with use of Equations 10 and 11.

There are other mechanisms which can introduce carbon atoms 4, 5, and 6 of the fructose-6-P. If this reaction is present, as would be expected, \(V_T\) will be overestimated. Estimations of \(V_T\) must therefore be considered maximum values.

Use of Glucose-2-\(^{14}C\) — Distributions in glycogen on incubation with glucose-2-\(^{14}C\) with beef adrenal cortex and thyroid slices, as already noted, have been reported (8, 9). Estimates of the pentose cycle contribution to glucose metabolism made in these tissues were based on the assumption of complete recycling, i.e., from the ratio of activity in C-1:C-2 with the use of the expression \(x_1/x_2 = 2/\text{PC} + 1\). Evidence for considerable, although perhaps not complete recycling, was obtained from the oxidation to \(^{14}CO_2\) of glucose-1-\(^{14}C\) and -6-\(^{14}C\) as compared to that from fructose-1-\(^{14}C\) and -6-\(^{14}C\). Estimates of \(V_T\) as shown by "Example 1," have now been made from the distribution of \(^{14}C\) reported for adipose tissue (4, 5), adrenal cortex (8), and thyroid (9) in carbon atoms 6 and 5 as well as 1 and 2 of glucose from glycogen with glucose-2-\(^{14}C\) as substrate. These are recorded in Table II. The pentose cycle contributions are within a few percent of those calculated by assuming complete recycling. The values of \(E_{-\text{x}}\) quantitate the extensive isotopic equilibration of fructose-6-P with glucose-6-P relative to glucose utilization. The values for thyroid are in accord with the report (12) that phosphohexose isomerase has high activity as compared to the activities of a large number of other enzymes, including glucokinase, when assayed in a preparation of thyroid cells from sheep. \(E_{X_T}\), the relative rate of the exchange reaction, has also been calculated. In these tissues, the contribution of this reaction to the incorporation of carbon of glyceraldehyde-3-P into glucose-6-P is greater than that of the pickup reaction which proceeds at the rate \(1/2\) PC. However, the rate of the exchange reaction is less than 0.3 times the rate of glucose utilization and much less than the rate of hexose-6-P isomerization. As already noted, estimates of \(E_{X_T}\) should be considered maximum because of the reactions, other
than the pickup reaction, that can introduce $^{13}$C of glucose-2-14C into carbon atoms 4, 5, and 6 of glucose-6-P.

Estimations remain dependent on the several assumptions previously enumerated (1, 2, 10); in particular, the existence of steady state conditions and single pools of intermediates. A major restriction is the limitations of the experimental procedures. The smaller the pentose cycle the less the quantity of $^{13}$C that will appear in carbon atoms 4, 5, and 6 via the pickup reaction. At low values of PC, the quantities may be too small for accurate measurement unless the exchange reaction or reversal or both introduce the carbon atoms of glyceraldehyde-3-P into hexose-6-P to a significant degree. In the tissues thus far examined, the exchange reaction does appear to be active as shown by Table II. In degradations of glucose-2-14C, $^{13}$C is found to a small extent in carbon atoms other than carbon 2 (9, 13) and the percentage of variations about the means in experiments is greater for carbon atoms with low activities (see Table II of Reference 4), so that the relative precision of the estimates is also affected.

**Use of Glucose-1-14C and -6-14C.**—The introduction of the transaldolase reactions into our previous model does not alter the equations derived for the estimation of the contribution of the pentose cycle to glucose metabolism with the use of glucose-1-14C and -6-14C as substrates and determining $^{13}$C incorporation into CO$_2$ and triose P or its derivatives. However, expressions previously presented (Tables I and II of Reference 1) for the distributions of $^{13}$C in the hexose 6-phosphates must be modified to take account of the transaldolase reactions. The effect of the pickup reaction on $x_1/x_6$ may be small, but small changes in $x_1/x_6$ when $E_{-h}$ is large can markedly change the estimate of PC when $E_{-h}$ is large can markedly change the estimate of PC.

$^\text{3}$

The provisions to write these 18 equations becomes apparent if Equations 1 to 12 are divided by 6 and Equations 13 to 18 by 3. Then any term of any equation represents the $^{13}$C atoms flowing either out of or into a pool at time $t$. For example, the first term on the right side of Equation 13 becomes $(\bar{y}_4/2)$. $y_4/2$ represents the flow of carbon regardless of the label of fructose-6-P converted to dihydroxyacetone-P. Only one-third of the carbon atoms in position 3 is $(\bar{y}_4/2)/3$. The fraction of carbon atoms in position 3 bearing label is $y_4$ and therefore the quantity of labeled carbon of fructose-6-P converted to dihydroxyacetone-P is $(\bar{y}_4/2)y_4$.

$^\text{4}$

State these equations are equated to zero. Certain relationships among the ratios of specific activities in the various positions are apparent. From Equations 4 and 5, the relationship

$$y_6/y_4 = x_6/x_4$$

holds. Eliminating $y_4$ and $y_5$ from Equations 10 and 11 with the aid of Equations 4 and 5, the result

$$x_4/x_5 = y_4/y_5$$

is obtained. With the use of Equations 4, 5, 10, and 11 to eliminate $y_4$, $y_5$, $z_1$, and $z_2$ in Equations 16 and 17, the relationship

$$x_4/x_5 = u_4/u_5$$

holds. Following similar substitutions in Equations 13 and 14,

$$u_4/u_5 = y_4/y_2$$

is found.

Through similar substitutions

$$x_6/x_5 = y_6/y_5 = z_6/z_2 = u_6/u_2 = y_6/y_2$$

also holds. Since Equations 1 to 3 and 7 to 9 are the same as Equations 12 to 17 in Reference 1 when a non-triose-P pathway is introduced, the relationships between $x_1/z_2$ and $y_1/y_2$ and the expressions for these ratios in terms of PC, $E_{-h}$, and NTP are the same as those given in Reference 1.

An equation showing the relationships between the ratios $x_2/z_4$ and $x_2/z_6$ is obtained easily by recognizing the algebraic identity $x_2/x_4 = (x_2/x_5) (x_1/x_6) / (x_1/x_2)$. From the relationships given by Equations 24 and 22 of Reference 1, the relationship

$$x_2/x_4 = x_1/x_5 = x_1/x_6$$

is found. As the equilibration of the hexose phosphates becomes complete ($E_{-h} \rightarrow \infty$),

$$x_2/x_4 = x_1/x_5$$

Through successive eliminations ($u_3$ from Equations 15 and 18, $z_3$ in the resulting equation and Equation 12, $y_3$ in the resulting equation and Equation 6, and $y_1$ in the resulting equation and Equation 1), an expression for $x_2/z_6$ is obtained. Expressing it in terms of PC, $E_{-h}$, NTP, $V_{al}/V_{l}$, and $EX_T$ where $EX_T = V_T/V_0$, the ratio becomes

$$x_2/z_6 = \frac{1}{x_2} PC + 2 EX_T \left[ \frac{PC (2-NTP)(1 + V_{al}/V_{l})}{(1-PC-NTP)(1 + 1/E_{-h})} - \frac{PC(1-NTP)(1 + V_{al}/V_{l})}{1-PC-NTP} + \frac{1}{1 + 1/E_{-h}} + \frac{2PC}{(1-NTP)(2 + V_{al}/V_{l}) \cdot 2PC} + 1 \right] + \frac{1}{(1-PC-NTP)(1 + 1/E_{-h})}$$

From this equation several conclusions are apparent. As the

$^\text{5}$

Intermediate expressions in this and succeeding derivations when complex are omitted from the text. They are available under Document No. 8576, from Chief, Photoduplication Service, Library of Congress, Washington 25, D. C., upon remittance of $1.25 for 35-mm microfilm or for photoprints.
extent of equilibration of the hexose phosphates increases, the ratio \( z_1/z_6 \) increases. At complete equilibration of the hexose phosphates, the value of \( z_1/z_6 \) is

\[
\frac{z_1}{z_6} = \frac{1}{PC + 2EX_T} \left[ \frac{PC(1 + \frac{V_6}{V_1})}{1-PC\cdotNTP} + 2 + \frac{V_6/V_1}{1-PC\cdotNTP} \right] + 1 + \frac{V_6/V_1}{1-PC\cdotNTP}
\]

(8')

The effect of an increase in the rate of isomerization of the triose phosphates is to decrease the ratio \( z_1/z_6 \). When the equilibration of the triose and hexose phosphates is complete (\( V_T \rightarrow \infty \) and \( E_{-h} \rightarrow \infty \)), the ratio is

\[
\frac{z_1}{z_6} = \frac{1}{PC + 2EX_T}\left[ \frac{PC}{1-PC\cdotNTP} + 2 \right] + \frac{2PC\cdotNTP}{1-PC\cdotNTP}
\]

(9')

The effect of a decrease in NTP while holding PC constant is to decrease the ratio \( z_1/z_6 \) (Equation 8' or 9').

In the application of these results to the estimation of pathways of glucocortic metabolism, \( E_{-h} \) and PC can be estimated from Equations 28 and 29, respectively, of Reference 1 where \( y_1/y_2 = z_4/z_6 \). With estimates of \( V_6/V_1 \) and NTP available, Equation 7' can be used to obtain an estimate of the rate of the transaldolase exchange reaction (see "Example 2"). In the following section, a method of estimating \( V_6/V_1 \) with the ratio of the specific yields of lactate or fatty acids will be derived.

Glucose-\(^{14}\)C and \(^{6}\)\(^{14}\)C as Substrates—When glucose contains
\(^{14}\)C in carbon atoms 1 and 6, the number of equations describing the change at time \( t \) in the \(^{14}\)C in the various positions in the four pools is reduced. In the model shown in Fig. 1, \(^{14}\)C can occur in carbon atoms 1 and 6 of the hexose-6-P pools when the substrate is glucose-\(^{14}\)C and in only carbon 3 of each of the two triose-P pools. When glucose-6-\(^{14}\)C is substrate, \(^{14}\)C occurs in only carbon 6 of the hexose-6-P pools and in carbon 3 of the triose-P pools. Expressions for \( z_1 \) and \( z_6 \) will be derived for two experimental situations. First, when two incubations are performed, one with glucose-\(^{14}\)C and the other with glucose-6-\(^{14}\)C and the specific activity of carbon 1 of the glucose-6-P or its derivative (\( z_1 \)) is determined from the first incubation and the specific activity of carbon 6 (\( z_6 \)) from the second incubation. Second, when a single incubation with glucose-1,6-\(^{14}\)C is performed, and \( x_1 \) and \( x_6 \) are determined from the isolated glucose-6-P pool and in carbon 3 of each of the two triose-P pools. When glucose-\(^{14}\)C is substrate, \(^{14}\)C occurs in only carbon 6 of the hexose-6-P pools and in carbon 3 of the triose-P pools. For these two situations the specific activity of carbon 1 of the glucose-6-P or its derivative (\( x_1 \)) is determined from the first incubation and the specific activity of carbon 6 (\( x_6 \)) from the second incubation. When two incubations are performed, the equations representing the change at time \( t \) in the \(^{14}\)C in the various carbon atoms of the four pools when glucose-1-\(^{14}\)C is substrate are

\[
\frac{dM_{x_1}}{dt} = V_6G_1 + V_{-a}y_1 - (V_1 + V_6 + V_a) x_1
\]

(10')

\[
\frac{dM_{x_6}}{dt} = V_{-a}y_6 - (V_1 + V_6 + V_a) x_6
\]

(11')

\[
\frac{dM_{y_1}}{dt} = V_6x_1 - (V_1 + V_6 + V_a) y_1
\]

(12')

\[
\frac{dM_{y_6}}{dt} = V_6x_6 + \frac{1}{2} V_1 (x_6 + z_6) + 2 V_{\tau_2} z_6 - (V_1 + V_6 + 2 V_T) y_6
\]

(13')

When glucose-6-\(^{14}\)C is substrate, these equations are the same as Equations 31 to 34 of Reference 5 with \( V_T = 0 \). Equations 10' and 12' are the same as Equations 2 and 3 of Reference 1 in which a non-triose-P pathway (\( V_4 \)) has been introduced and glucose is sole substrate (\( g = 1 \)). Therefore, the expressions for \( x_1 \) and \( y_1 \) in terms of PC, \( E_{-h} \), and NTP are those given in Table I of Reference 1 with the modification for the presence of a non-triose-P pathway. By an elimination procedure previously described, the expression for \( x_6 \) when glucose-6-\(^{14}\)C is substrate is

\[
\frac{dM_{x_6}}{dt} = \frac{1}{2} V_6y_6 + \frac{1}{2} V_1 x_6 + V_{\tau_6} + V_{\eta_6} - (\frac{1}{2} V_1 + V_T + V_6 + V_a) z_6
\]

(14')

When glucose-6-\(^{14}\)C is substrate, these equations are the same as Equations 31 to 34 of Reference 5 with \( V_T = 0 \).

The complicated fraction inside the braces is positive for all of the values of PC, \( E_{-h} \), \( E_{x_6} \), and NTP, and hence, \( x_6 \leq G_6 \). In the absence of the transaldolase reactions, the expression for \( x_6 \) which can be obtained from Table I of Reference 1 is \( x_6 = G_6 \). Since the expression for \( x_6 \) is unchanged, the ratio \( x_1/x_6 \) is increased by the presence of transaldolase reactions. As can be seen by sample calculations, their effects on the estimation of PC can be considerable when the ratio \( x_1/x_6 \) is used to estimate PC without an adjustment being made for their presence.

If there is no non-triose-P pathway (NTP = 0), the specific activities of dihydroxyacetone-P and glyceraldehyde-3-P are unaltered by the introduction of the transaldolase reactions. When glucose-6-\(^{14}\)C is substrate, this result becomes apparent by equating Equations 31, 32, and 34 of Reference 5 to zero (isotopic steady state), setting both \( V_2 \) and \( V_4 \) equal to zero and adding. The sum of these three equations is

\[
V_6G_6 + 2V_\eta_6 = 2(V_\eta - V_a) z_6
\]

(17')

and is similar to the equation obtained when the transaldolase reactions are assumed to be absent. Equation 33 of Reference 5 is identical with that obtained when the reactions are assumed to be absent (2). Hence, the specific activities of \( u_4 \) and \( z_4 \) are unchanged by the presence of the reactions. Reasoning is similar when glucose-1-\(^{14}\)C is substrate. The additional fact that the specific activity of carbon 1 of fructose-6-P is independent of the presence of the reactions must be remembered in showing that the specific activities of \( u_4 \) and \( z_4 \) are unchanged.

Since the specific activities of dihydroxyacetone-P and glyceraldehyde-3-P are unaffected by the presence of the transaldolase reactions, an estimate of \( V_6/V_1 \) can be obtained from the ratio of the specific yields of glyceraldehyde-3-P or a derivative reflecting its activity such as fatty acids or lactate (2). By eliminating \( u_4 \) from Equations 38 and 17',

\[
V_6G_6 = 2[V_\eta + V_p - V_\eta/(1 + V_6/V_1)] z_6
\]

(18')

is obtained. The specific yield of \(^{14}\)C in the products from carbon 3 of glyceraldehyde-3-P, when glucose-6-\(^{14}\)C is substrate, equals \( V_{\eta_6}/V_6G_6 \), where \( z_6 \) is given by Equation 18'. By adding Equations 11', 13', and twice Equation 15',

\[
2V_{\eta_6} = 2(V_\eta + V_p) z_6
\]

(19')
results. By adding Equations 10', 12', and twice Equation 14' and eliminating \( u_1 \) from the sum and Equation 19', Equation 20' results.

\[
(V_G G_1 - V_G x_2)/(1 + V_G t/V_1) = 2 \left[ V_{-t} + V_{+t} - V_{-t}/(1 + V_G t/V_1) \right] z_k
\]  

(20')

When glucose-1-\(^1\)C is substrate, the specific yield in the products from carbon 3 of glyceraldehyde-3-P equals \( V_G x_3/V_0 G_1 \), where \( x_3 \) is given by Equation 20'. Forming the ratio of the specific yields from glucose-1-\(^1\)C and -6-\(^1\)C, which has been designated \( \gamma (2) \), by forming the ratio of Equation 20' to Equation 18' and solving for \( 1 + V_G t/V_1 \)

\[
1 + V_G t/V_1 = (G_1 - 3 P C x_1)/\left(\gamma G_k\right)
\]  

(21')

where from Table I of Reference 1

\[
x_1 = \frac{[1 + (1-PC-NTP)/E_c]G_1}{1 + 2 PC + (1-PC-NTP)/E_c}
\]

When glucose-1,6-\(^1\)C is substrate, the 6 equations, 10', 12', 14', 15' and 31, and 32 of Reference 5 describe the change at time \( t \) in the quantity of \(^1\)C in the four pools of the system. After the derivation used when two separate incubations are performed, an expression for \( x_4 \) may be obtained. The expression for the specific activity of carbon 6 of glucose-6-P is given by Equation 16' to which the term

\[
-\frac{(PC + 2 E_r)(1 PC NTP) y_1}{(1 + V_G t/V_0 G_6)}
\]  

(22')

is added to the numerator of the fraction, where \( y_1 \) represents the specific activity of carbon 1 of fructose-6-P. The expressions for \( y_1 \) are given in Table I of Landau et al. (1) with the appropriate modification for the presence of a non-triose-P pathway. As for separate incubations, the specific activities of carbon 1 of glucose-6-P and fructose-6-P are unaltered by the presence of a non-triose-P pathway.

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Bernard R. Landau and Glenn E. Bartsch


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