It has been shown by Newsholme & Crabtree (1976) that the rate of fructose 6-phosphate/fructose 1,6-bisphosphate cycling (C) is given by the equation

\[ C = \frac{T(1-S_1)}{(S_1-S_2)} \]

where \( T \) is the glycolytic rate, \( S_1 = (\text{3H/14C}) \) ratio of hexose monophosphates/(\text{3H/14C}) ratio of glucose and \( S_2 = (\text{3H/14C}) \) ratio of fructose 1,6-bisphosphate/(\text{3H/14C}) ratio of glucose.

This formula is derived for a system such as that described in Scheme 1. However, in the main paper (Challiss et al., 1984), it is clear that glycogenolysis and glycolysis may occur under the various conditions investigated. Thus the system investigated is better described by Scheme 2. It is therefore necessary to derive new equations to take glycogen/glucose 1-phosphate cycling into account.

From Scheme 2, let \( J \) denote the rate of glycolysis, \( F \) and \( C \) the fluxes through 6-phosphofructokinase and fructose-1,6-bisphosphatase respectively, \( A \) the rate of glycogenolysis and \( B \) the rate of glycogen synthesis. Let the specific radio-activities of glucose, hexose monophosphate (HMP) and fructose 1,6-bisphosphate (FBP) be \( S_g, S_h \) and \( S_f \) for \( ^{3}H \) and \( R_g, R_h \) and \( R_f \) for \( ^{14}C \) respectively.

It is assumed that glycogen acts as a ‘sink’ (i.e. no \( ^{14}C \) flux comes from glycogen to enter the hexose monophosphate pool).

Considering \( ^{14}C \) fluxes in the steady state:

\[ GR_g + CR_f = Rh (F+B) \]  

Since the \( ^{14}C \) label is not diluted between HMP and FBP,

\[ Rh = R_f \]

and eqn. (i) becomes

\[ GR_g + CR_h = Rh (J+B) \]

\[ GR_g = Rh (J+B) \]

\[ R_h/R_g = \frac{G}{(J+B)} \]  

Glucose \( \xrightarrow{G} \) HMP \( \xrightarrow{F} \) FBP \( \xrightarrow{T} \) Lactate

Scheme 1. Model for substrate cycling at the level of fructose 6-phosphate/fructose 1,6-bisphosphate, assuming that the hexose monophosphate pool is undiluted by glycogenolysis.

HMP represents hexose monophosphate, FBP represents fructose 1,6-bisphosphate, and \( F, C \) and \( T \) represent the fluxes through 6-phosphofructokinase, fructose 1,6-bisphosphatase and glycolysis respectively.

Glucose \( \xrightarrow{G} \) HMP \( \xrightarrow{F} \) FBP \( \xrightarrow{J} \) Lactate

Scheme 2. Model for substrate cycling at the level of fructose 6-phosphate/fructose 1,6-bisphosphate, taking account of an active glycogen/glucose 1-phosphate cycle.

HMP represents hexose monophosphate, FBP represents fructose 1,6-bisphosphate, \( G, F, C \) and \( J \) represent the fluxes through glucose phosphorylation, 6-phosphofructokinase, fructose 1,6-bisphosphatase and glycolysis respectively, and \( A \) and \( B \) represent the fluxes between glycogen and HMP.
Let the rates of $^{14}C$ incorporation into lactate and glycogen be $\lambda$ and $\gamma$ respectively.
\[
\lambda = JR_l = JR_h
\]
\[
\gamma = BR_h
\]
Thus
\[
\lambda/\gamma = J/B
\]
So that
\[
B = J \times \gamma/\lambda \tag{2}
\]
Let us now consider $^3H$ fluxes in the steady state.
Flux to HMP = flux from HMP
\[
GS_g + CS_f = S_h (F + B)
\]
Dividing through by $S$, and letting $S_f/S_g = r_2$ and $S_h/S_g = r_1$:
\[
G + Cr_2 = (J + C + B) \quad r_1 \tag{3}
\]
where $r_1$ is $^3H$ sp. radioactivity in HMP/$^3H$ sp. radioactivity in glucose
\[
= \frac{^3H \text{ in HMP}}{|\text{HMP}|} \quad \frac{^3H \text{ in glucose}}{|\text{glucose}|} \tag{4}
\]
The concentration terms can be eliminated from the ratios by using the $^{14}C$ sp. radioactivities:
\[
R_h = ^{14}C \text{ in HMP} / |\text{HMP}|
\]
\[
R_g = ^{14}C \text{ in glucose} / |\text{glucose}|
\]
Substituting $R_h$ and $R_g$ into eqn. (4):
\[
r_1 = \frac{(^{13}H/^{14}C) \text{ in HMP}}{(^{13}H/^{14}C) \text{ in glucose}} \times \frac{R_h}{R_g}
\]
By using the definitions of $S_1$ and $S_2$ given above, thus:
\[
r_1 = S_1 \times R_h/R_g
\]
\[
r_2 = S_2 \times R_h/R_g
\]
and from eqn. (1):
\[
r_1 = \frac{S_1G}{J + B}
\]
\[
r_2 = \frac{S_2G}{J + B}
\]
from eqn. (3):
\[
G + Cr_2 = (J + C + B) \quad r_1
\]
Substituting for $r_1$ and $r_2$:
\[
G + \frac{CGS_2}{J + B} = \frac{(J + C + B) S_1G}{J + B}
\]
multiplying by $(J + B)G$:
\[
(J + B) + CS_2 = (J + C + B)S_1
\]
Rearranging:
\[
C = \frac{(J + B) (1 - S_1)}{(S_1 - S_2)} \quad \text{[from eqn. (2)]}
\]
Thus the rate of cycling can be calculated from the total flux (glucose plus glycogen) to lactate, the $[^{14}C]$glucose flux to lactate, the $[^{14}C]$glucose flux to glycogen and the $^3H/^{14}C$ ratios in glucose, HMP and FBP.
Moreover, the actual fluxes $A$ (glycogen to HMP), $B$ (HMP to glycogen) and $G$ (glucose to HMP) may also be estimated from an experimentally determined parameter. Thus, from eqn. (2):
\[
B = J \times \gamma/\lambda
\]
and as $\lambda = JR_h$ and thus, from eqn. (1)
\[
G = \frac{J + B}{JR_g} \times \lambda
\]
And from the steady-state flux balance:
\[
A = B + (J - G)
\]