

- Rieder, S. V. & Rose, I. A. (1959) *J. Biol. Chem.* **234**, 1007-1010
 Rose, I. A. & O'Connell, E. L. (1960) *Biochim. Biophys. Acta* **42**, 159-160

- Surholt, B. & Newsholme, E. A. (1981) *Biochem. J.* **198**, 621-629
 Surholt, B. & Newsholme, E. A. (1983) *Biochem. J.* **210**, 49-54

APPENDIX

Calculation of the rate of fructose 6-phosphate/fructose 1,6-bisphosphate cycling in a tissue with active glycogenolysis and/or glycogen synthesis

R. A. John CHALLISS,* Bernard CRABTREE† and Eric A. NEWSHOLME*

*Department of Biochemistry, University of Oxford, South Parks Road, Oxford OX1 3QU, U.K., and

†The Rowett Research Institute, Greenburn Road, Bucksburn, Aberdeen AB2 9SB, Scotland, U.K.

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, $S_1 = (^3\text{H}/^{14}\text{C})$ ratio of hexose monophosphates/ $(^3\text{H}/^{14}\text{C})$ ratio of glucose and $S_2 = (^3\text{H}/^{14}\text{C})$ ratio of fructose 1,6-bisphosphate/ $(^3\text{H}/^{14}\text{C})$ 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 glycogen synthesis 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 radioactivities of glucose, hexose monophosphate (HMP) and fructose 1,6-bisphosphate (FBP) be S_g , S_h and S_f for ^3H 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:

$$\text{Flux to HMP} = \text{flux from HMP} \quad (\text{i})$$

$$GR_g + CR_f = R_h (F + B)$$

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

$$R_h = R_f$$

and eqn. (i) becomes

$$GR_g + CR_h = R_h (F + B)$$

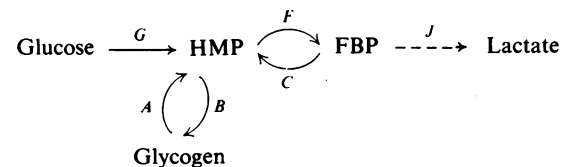
$$GR_g = R_h (J + B)$$

$$R_h/R_g = \frac{G}{(J + B)} \quad (1)$$



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.



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 λ and γ respectively.

$$\lambda = JR_f = JR_h$$

$$\gamma = BR_h$$

Thus

$$\lambda/\gamma = J/B$$

So that

$$B = J \times \gamma/\lambda \quad (2)$$

Let us now consider ^3H fluxes in the steady state.

Flux to HMP = flux from HMP

$$GS_g + CS_f = S_h (F + B)$$

$$= S_h (J + C + B)$$

Dividing through by S , and letting $S_f/S_g = r_2$ and $S_h/S_g = r_1$:

$$G + Cr_2 = (J + C + B) r_1 \quad (3)$$

where r_1 is ^3H sp. radioactivity in HMP/ ^3H sp. radioactivity in glucose

$$= \frac{^3\text{H in HMP}/[\text{HMP}]}{^3\text{H in glucose}/[\text{glucose}]} \quad (4)$$

The concentration terms can be eliminated from the ratios by using the ^{14}C sp. radioactivities:

$$R_h = ^{14}\text{C in HMP}/[\text{HMP}]$$

$$R_g = ^{14}\text{C in glucose}/[\text{glucose}]$$

Substituting R_h and R_g into eqn. (4):

$$r_1 = \frac{(^3\text{H}/^{14}\text{C}) \text{ in HMP}}{(^3\text{H}/^{14}\text{C}) \text{ in glucose}} \times R_h/R_g$$

By using the definitions of S_1 and S_2 given above, thus:

$$r_1 = S_1 \times R_h/R_g$$

$$r_2 = S_2 \times R_f/R_g$$

and from eqn. (1):

$$r_1 = \frac{S_1 G}{J + B}$$

$$r_2 = \frac{S_2 G}{J + B}$$

from eqn. (3):

$$G + Cr_2 = (J + C + B) r_1$$

Substituting for r_1 and r_2 :

$$G + \frac{CGS_2}{J + B} = \frac{(J + C + B) S_1 G}{J + B}$$

multiplying by $(J + B)/G$:

$$(J + B) + CS_2 = (J + C + B)S_1$$

Rearranging:

$$C = \frac{(J + B)(1 - S_1)}{(S_1 - S_2)}$$

$$= \frac{J(1 + \gamma/\lambda)(1 - S_1)}{(S_1 - S_2)} \quad \text{[from eqn. (2)]}$$

Thus the rate of cycling can be calculated from the total flux (glucose plus glycogen) to lactate, the $[^{14}\text{C}]$ glucose flux to lactate, the $[^{14}\text{C}]$ glucose flux to glycogen and the $^3\text{H}/^{14}\text{C}$ 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:

$$A = B + (J - G)$$

Challiss, R. A. J., Arch, J. R. S. & Newsholme, E. A. (1984) *Biochem. J.* **221**, 153-160
Newsholme, E. A. & Crabtree, B. (1976) *Biochem. Soc. Symp.* **41**, 61-109