The role of high rates of glycolysis and glutamine utilization in rapidly dividing cells

E. A. NEWSHOLME, B. CRABTREE and M. S. M. ARDAWI

Department of Biochemistry, University of Oxford, South Parks Road, Oxford OX1 3QU, England; Rowett Research Institute, Aberdeen, Scotland; and Department of Biochemistry, College of Medicine and Allied Sciences, P.O. Box 9029, King Abdulaziz University, Jeddah 21413, Kingdom of Saudi Arabia

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The rates of utilization of both glucose and glutamine are high in rapidly dividing cells such as enterocytes, lymphocytes, thymocytes, tumour cells; the oxidation of both glucose and glutamine is only partial, glucose to lactate and glutamine to glutamate, alanine or aspartate; and these partial processes are termed glycolysis and glutaminolysis respectively. Both processes generate energy and also provide precursors for important biosynthetic processes in such cells. However, the rates of utilization of precursors for macromolecular biosynthesis are very low in comparison to the rates of partial oxidation, and energy generation per se may not be the correct explanation for high rates of glycolysis and glutaminolysis in these cells since oxidation is only partial and other fuels can be used to generate energy. Both the high fluxes and the metabolic characteristics of these two processes can be explained by application of quantitative principles of control as applied to branched metabolic pathways (Crabtree & Newsholme, 1985). If the flux through one branch is greatly in excess of the other, then the sensitivity of the flux of the low-flux pathway to regulators is very high. Hence, it is suggested that, in rapidly dividing cells, high rates of glycolysis and glutaminolysis are required not for energy or precursor provision per se but for high sensitivity of the pathways involved in the use of precursors for macromolecular synthesis to specific regulators to permit high rates of proliferation when required - for example, in lymphocytes in response to a massive infection.

The ability of some cells to convert glucose to lactate at a very high rate was observed in the 1920s by Otto Warburg (see Warburg, 1956a). For example, rapidly growing ascites tumour cells are able to convert an amount of glucose equal to 30% of their dry weight to
lactate per hour (Warburg, 1956b). This high rate of glycolysis is also observed in other rapidly dividing cells, e.g. absorptive cells of the small intestine (Hanson & Parsons, 1977), in the chorion during the first few days of embryonic development (Warburg, 1956b) and in both resting and stimulated lymphocytes (Roos & Loos, 1973; Hume et al., 1978). In these rapidly dividing cells very little if any glucose is oxidized to carbon dioxide; almost all of it is converted into lactate. This finding led Warburg to suggest that a defect in oxidation metabolism could be a cause of malignancy. However, there is no doubt that rapidly dividing cells possess an effective aerobic system and recent work with lymphocytes has demonstrated that, although they do not oxidize much glucose, other fuels can be oxidized (e.g. glutamine, ketone bodies, fatty acids - see Ardawi & Newsholme, 1985a, for review.) Not surprisingly, the significance of the high rate of glucose utilization in rapidly dividing cells has been debated since the 1920s and several proposals have been put forward. First, since it provides the precursors for a number of macromolecules that are important during cell division (e.g. glucose 6-phosphate is the substrate for the pentose phosphate pathway that provides reducing power and ribose for RNA and DNA synthesis, and glycerol phosphate is essential for the formation of phospholipids), it has been suggested that a high capacity is necessary to provide precursors at a sufficient rate for the biosynthetic processes; however, the maximal rate of utilization of the glycolytic intermediates for biosynthetic purposes is only a few percent of the rate of glycolysis (Hume & Weidemann, 1979). Secondly, the high rate of glycolysis is necessary to maintain high concentrations of glycolytic intermediates for biosynthesis (Hume & Weidemann, 1979; Morgan & Faik, 1981), but the concentrations of glycolytic intermediates in rapidly dividing cells are not noticeably higher than in non-dividing cells such as muscle or liver (see Williamson et al., 1970, for tumour cells; Ardawi & Newsholme, 1983, for lymphocytes; Brosnan & Williamson, 1974, for other cells). Thirdly, the genetic changes that are responsible for malignancy also coincidently increase the glycolytic capacity (see Newsholme & Leech, 1983). However, in a similar manner to glycolysis, another pathway that provides precursors for biosynthetic processes also has a high capacity in rapidly dividing cells. This is the utilization of glutamine (see Lund, 1980; Krebs, 1980; McKeenan, 1982; Kovacevic & McGivan, 1983). A high rate of glutamine utilization occurs in the absorptive cells of the small intestine which are also rapidly dividing (Windmueller & Spaeth, 1974).

It has been known for some time that glutamine is important in rapidly dividing cells (for review, see Krebs, 1980; McKeenan, 1982; Kovacevic & McGivan, 1983) and this is not surprising since it provides nitrogen for a number of important precursors involved in the synthesis of macromolecules, including purines and pyrimidines. What is surprising is the very high rate of glutamine utilization by rapidly dividing cells: rates of utilization of approx. 11.0, 3.7, 0.6 and 2.7 μmol/min per g dry wt. have been reported for enterocytes, colonocytes, thymocytes and lymphocytes respectively (Watford et al., 1979; Roediger, 1982; Brand et al., 1984; Ardawi & Newsholme, 1983). These rates are similar to or greater than the rates of glucose utilization by these cells (see Ardawi & Newsholme, 1983); indeed for lymphocytes, the rate of glutamine utilization is four-fold greater
than that of glucose. As for glycolysis, it has been shown that, of
the rate of glutamine utilization by lymphocytes, the maximum
proportion of this that could be used in provision of precursors for
RNA and DNA synthesis is only 4% (Ardawi & Newsholme, 1984a).
Hence, it has been suggested that the reasons for the high rate of
glutamine utilization for rapidly dividing cells is energy provision
(Krebs, 1980; Ardawi & Newsholme, 1982; McKeehan, 1982; Kovacevic
& McGivan, 1983). However, although it does undoubtedly provide
energy, there are several problems with this suggestion. First, studies
on rapidly dividing cells including lymphocytes show that most of the
carbon of glutamine is converted to glutamate, aspartate and lactate
and very little if any is completely oxidized via acetyl-CoA and the
Krebs cycle (Ardawi & Newsholme, 1985a). Thus, glutamine is only
partially oxidized and, because of the similarity to glycolysis, this
partial oxidative pathway has been termed 'glutaminolysis' (McKeehan,
1982). If the role of glutaminolysis was solely to provide energy, it
would be expected that more of its carbon would be completely
oxidized by the Krebs cycle. Secondly, it has been shown that
lymphocytes can oxidize ketone bodies or long chain fatty acids
(Ardawi & Newsholme, 1984b), colonocytes can utilize butyrate or
ketone bodies (Ardawi & Newsholme, 1985b) and enterocytes can use
ketone bodies (see Parsons, 1979), so why cannot the rate of fatty
acid or ketone body oxidation be increased to provide more energy and
decrease the demand for glutamine. The requirement for glutamine by
malignant cells is well established (see Lund, 1980; Krebs, 1980;
McKeehan, 1982) but the question of why glutamine is preferentially
used as an energy source for these cells has also been raised (see
Kovacevic & McGivan, 1983).

An important characteristic of all rapidly dividing cells is that the
rates of both glycolysis and glutaminolysis are high and that both
pathways provide vitally important precursors for macromolecular
requirement in cell division. This similarity led to a search for a
common characteristic that would explain why high rates for such
pathways were necessary in rapidly dividing cells. An answer was
forthcoming from the application of the recently developed quantita-
tive principles of metabolic control put forward by Crabtree and

**Regulatory Analysis of Branched Systems**

A branched pathway can be represented as follows:

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   X  \( \sim \) (J)
 A -------> B -------> (J_a)
      E_1        E_2        (J_b)
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In this system there are three fluxes, J, J_a and J_b, such that J = (J_a + J_b); X is a regulator of E_2 and, since there is no direct feedback
from E_2 to E_1, X changes J_a at the expense of J_b, leaving J unchanged. Let us assume that J_a represents a biosynthetic pathway
for which B is a precursor (e.g. J_a could represent the generation of
pentose phosphate for DNA/RNA synthesis and B the precursor glucose 6-phosphate). As $J_a$ is increased (by regulator X) the concentration of B will tend to decrease and 'deflect' flux from $J_b$ to $J_a$. However, assuming that $E_2$ is not saturated with B, this decreased concentration of B will also reduce the flux through $E_2$ and hence reduce $J_a$. Consequently, the increased $J_a$ brought about by regulator X will be 'opposed' by the decreased concentration of B, resulting in a less effective response of $J_a$ to X.

These arguments can be quantified by expressing the rate equations as 'power' approximations (see Crabtree and Newsholme, 1985):

for $E_2$,

$$J_a = k[B] \frac{S_i(B)}{S_i(X)}$$  (1)

for $E_3$,

$$J_b = k[B] \frac{S_i(B)}{S_i(X)}$$  (2)

Here $k$ is an arbitrary constant of integration and $S_i$ denotes an intrinsic sensitivity: for example $S_i(E_2(X))$ measures the response of $E_2$ to X when B remains constant (for further details, see Crabtree & Newsholme, 1985). These equations can be combined to eliminate [B] and produce an equation involving only $J_a$ and [X]: the index of [X] in this equation will then represent the net sensitivity of $J_a$ to X; i.e. the response to X in situ, when B also varies. However, to do this the term $J_b$ must also be eliminated and this can be achieved by using the balance equation for the fluxes:

$$J_a + J_b = J.$$

This can also be expressed as a power approximation (see Crabtree & Newsholme, 1985)

$$J_a J_a J_b J_b = k J^2$$

which, since J is not affected by X (i.e. there is no direct feedback from $E_2$ to $E_1$) becomes:

$$J_a J_a J_b J_b = k$$

whence

$$J_b = k J_a - J_a/J_b.$$

Therefore equation 2 can be written as:

$$J_a - J_a/J_b = k [B] S_i(B)$$

so that

$$[B] = k J_a - J_a/(J_b S_i(B)).$$
Replacing $[B]$ by this function of $J_a$, equation 1 becomes:

$$J_a = k[X] S_i(X) \cdot J_a \frac{E_2 E_3}{-J_a S_i(B)/(J_b \cdot S_i(B))}$$

which, after re-arranging the terms and indices, becomes:

$$J_a = k[X] \frac{E_2 S_i(X) \cdot S_i(B) J_b}{J_b S_i(B) + J_a S_i(B)}$$

The index of $(X)$ is the net sensitivity of $J_a$ to $X$, $S(X)$, so that

$$S_a = \frac{E_2 S_i(X) \cdot S_i(B) J_b}{J_b S_i(B) + J_a S_i(B)}$$

or

$$S_a = \frac{E_2 S_i(X) \cdot S_i(B) (J_b/J_a)}{E_3 (J_b/J_a) + S_i(B)}$$

where $S_i(X)$ is the intrinsic sensitivity of $E_2$ to regulator $X$ and $E_2$, $E_3$, $S_i(B)$ are the intrinsic sensitivities to $B$ of reactions $E_2$ and $E_3$ respectively. This equation shows that, as the ratio $J_b/J_a$ increases, the sensitivity of $J_a$ to $X$ increases from zero to a value approaching $E_2 S_i(X)$, i.e., the intrinsic sensitivity of $E_2$ (and hence $J_a$) to $X$. Since the intrinsic sensitivity is that observed in the absence of 'oppositions' to the action of $X$ in situ (see Crabtree & Newsholme, 1985), the overall response of $J_a$ to $X$ in situ is most effective when $J_b$ is much greater than $J_a$, i.e., when $J_a$ is much smaller than the total flux, $J$. Under these conditions the biosynthetic pathway ($J_a$) does not become seriously limited by changes in precursor concentration during the 'deflection' of flux from $J_b$ to $J_a$ and is therefore most sensitive to the action of regulator $X$.

**Application of the Branched-Pathway-Sensitivity Principle to Glycolysis and Glutaminolysis in Rapidly Dividing Cells**

The principle described above is relevant to the discussion of glycolysis and glutaminolysis in rapidly dividing cells. Both pathways exhibit high rates and both pathways provide precursors for macromolecular synthesis, but the rates of the latter processes are very small in comparison. Hence the situation can be represented as follows:

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  glucose  precursors  macromolecules
  glutamine  end-products
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end-products
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precursors
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macromolecules
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  glucose  precursors  macromolecules
  glutamine  end-products
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and the condition for high sensitivity for the biosynthetic branch is provided (see above). The physiological significance of this high sensitivity is that the rate of macromolecular synthesis ($J_3$) can be markedly increased without decreasing significantly the concentration of the metabolic intermediates of the main pathway ($B$) which would 'oppose' the stimulation of the pathway for macromolecular synthesis. The only other way of providing such a sensitive feedback system would be for the 'precursor' ($B$) to 'communicate' directly (e.g. by feedback inhibition) with the flux-generating step of the overall pathway (i.e. $E_1$) so that an increase in demand for macromolecular synthesis would be met by increasing total flux ($J$). However, this would require a very sensitive response of the flux-generating step to the precursor (or a related metabolite); otherwise the precursor concentration would fall to values that could seriously 'oppose' and hence limit the response to an increased demand for macromolecular synthesis. Although high sensitivity can be provided at control sites (see Newsholme & Leech, 1983), they would probably be of limited value in the above system because the flux-generating steps for both glycolysis-from-glucose and glutaminolysis are not in the same tissue as these 'pathways'; the flux-generating step for glycolysis-from-glucose is probably at hepatic phosphorylase and that for glutaminolysis is probably the release of glutamine from muscle (see Newsholme & Leech, 1983). Thus, even if a sensitive feedback effect of precursor (or a related metabolite) on the flux-generating step was developed, it could only operate by providing a higher concentration of glutamine or glucose in the bloodstream and this would increase the rate of utilization of these compounds by other tissue and so interfere in the supply of these key intermediates for the specific rapidly dividing cells. Such a complex (and potentially ineffective) direct feedback control mechanism is not required if cells maintain high rates of glycolysis and glutaminolysis, for reasons given above. Although at first sight this may appear wasteful, it provides a relatively simple and effective control system that can permit macromolecular synthesis and hence cell division to occur whenever required for essential purposes in the animal; for example, cell division in the small intestine is essential for digestion and cell division in lymphocytes is essential in the immune response.

It should be pointed out that there are several problems with the hypothesis proposed above. First, mutant cells which were deficient in phosphoglucoisomerase or phosphoglycerate kinase, so that the rate of lactate formation was very low, not only survived but also proliferated and exhibited malignant properties in the case of transformed cells (see Morgan & Faik, 1981; McKeehan, 1982, for reviews) that is, that low rates of glycolysis are not required for proliferation. However, it can be argued that such cells might adapt to the low activity of these enzymes by an increase in flux through the pentose phosphate pathway which would produce the biosynthetic precursors required, including pentose sugars and reducing power, and also accumulate glycerol phosphate (from triose phosphate) for phospholipid synthesis and perhaps release glycerol instead of lactate. Secondly, for both normal and cultured cells, in vitro, limitation of glucose to such an extent that lactate production was markedly decreased did not decrease the rate of proliferation in proportion to that of lactate (see McKeehan,
1982, for review). Nonetheless, the rate of proliferation was affected so that we suggest that high rates of flux through both glycolysis and glutaminolysis are required to ensure high rates of proliferation. If cell division is essential for the well-being of the organism, under some conditions very high rates of proliferation may be essential for survival (e.g. in the immune system in response to a massive infection). The high rates of glycolysis and glutaminolysis normally observed in such cells might, therefore, be considered a fail-safe system to ensure sufficient sensitivity in biosynthetic pathways to provide adequately for macromolecular synthesis. Thirdly, it has been suggested that although both glycolysis and glutaminolysis are required for proliferation, high fluxes may only be required in one pathway. Thus it has been observed in HeLa cells (Reitzer et al., 1979) and human diploid cells (Zielke et al., 1978) that the presence of a high concentration of glucose decreases the rate of glutaminolysis and vice versa. However, in detailed studies on lymphocyte metabolism under conditions in which the ATP/ADP and NAD/NADH concentrations were maintained, the opposite effect was observed; that is, the presence of glucose and glutamine in the incubation medium enhanced the rates of both glycolysis and glutaminolysis (Ardawi & Newsholme, 1983), suggesting an important role for high fluxes of the two pathways.

It remains to be seen how many other branched metabolic pathways have similar characteristics to glycolysis and glutaminolysis in rapidly dividing cells. A possible example is the rate of utilization of ketone bodies by neonatal brain. This is known to be high, and the significance of this has been considered to be the provision of precursors for lipid formation for myelination (Williamson & Buckley, 1973). However, the rate of utilization of ketones for lipogenesis is very small in comparison to the oxidation of ketone bodies by neonatal brain (Williamson, 1975). This is precisely the condition necessary to ensure a high sensitivity of the lipogenic process, from acetyl-CoA to fatty acid, to specific regulators within the brain (see above).

References
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