Aerobic Glycolysis and Lymphocyte Transformation

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1. The role of enhanced aerobic glycolysis in the transformation of rat thymocytes by concanavalin A has been investigated. Concanavalin A addition doubled $[U-^{14}C]$ glucose uptake by rat thymocytes over 3 h and caused an equivalent increased incorporation into protein, lipids and RNA. A disproportionately large percentage of the extra glucose taken up was converted into lactate, but concanavalin A also caused a specific increase in pyruvate oxidation, leading to an increase in the percentage contribution of glucose to the respiratory fuel. 2. Acetoacetate metabolism, which was not affected by concanavalin A, strongly suppressed pyruvate oxidation in the presence of $[U-^{14}C]$ glucose, but did not prevent the concanavalin A-induced stimulation of this process. Glucose uptake was not affected by acetoacetate in the presence or absence of concanavalin A, but in each case acetoacetate increased the percentage of glucose uptake accounted for by lactate production. 3. $[^3H]$ thymidine incorporation into DNA in concanavalin A-treated thymocyte cultures was sensitive to the glucose concentration in the medium in a biphasic manner. Very low concentrations of glucose (25 μM) stimulated DNA synthesis half-maximally, but maximum $[^3H]$ thymidine incorporation was observed only when the glucose concentration was raised to 1 mM. Lactate addition did not alter the sensitivity of $[^3H]$ -thymidine uptake to glucose, but inosine blocked the effect of added glucose and strongly inhibited DNA synthesis. 4. It is suggested that the major function of enhanced aerobic glycolysis in transforming lymphocytes is to maintain higher steady-state amounts of glycolytic intermediates to act as precursors for macromolecule synthesis.

Since the original observation that aerobic glycolysis is enhanced in malignantly transformed cells (Warburg, 1956), there has been considerable interest in the role of glycolysis in the control of cell proliferation. One of the earliest events in virus-induced transformation of mammalian cells is an increase in glycolysis (Hatanaka, 1974; Plagemann & Richey, 1974) that is due to a stimulation of plasma-membrane glucose transport (Weber, 1973; Venuta & Rubin, 1973; Bissell, 1976). Wang *et al.* (1976) have suggested that enhanced aerobic glycolysis is not unique to malignantly transformed cells, but is, instead, a requirement for normal cell proliferation. Proof of this proposal is seen in the case of mitogenically transformed lymphocytes, which produce lactate at an accelerated rate without any diminution of O_2 consumption (Cooper *et al.*, 1963; Roos & Loos, 1973; Culvenor & Weidemann, 1976; Hedeskov, 1968). In cultured mouse spleen (Wang *et al.*, 1976) and rat thymus lymphocytes (Schweinberger,

1976) the rate of aerobic lactate production may increase more than 20-fold after 50 h in the presence of the plant lectin concanavalin A. Concanavalin A-stimulated glycolysis probably results from a stimulation of plasma-membrane glucose transport (Yasmeen *et al.*, 1977; Whitesell *et al.*, 1977), which Yasmeen *et al.* (1977) have proposed is rate-limiting for thymocyte glycolysis. Unlike malignantly-transformed cells, concanavalin A-treated lymphocytes undergo a discrete proliferative response, after which lactate production returns to basal values (Wang *et al.*, 1976).

Mitogen-induced lymphocyte transformation may be a useful normal control system for distinguishing between those aspects of tumour growth associated with controlled cell proliferation and those associated with malignancy. In the present paper we report a quantitative assessment of the fate of carbon from $[U-^{14}C]$ glucose in control and mitogenically transformed rat thymus lymphocytes. To assess the role of enhanced aerobic glycolysis in the initiation of DNA synthesis, we have measured the effect of glucose concentration in the medium on $[^3H]$ thymidine incorporation into DNA in concanavalin A-treated lymphocyte cultures.

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Materials and Methods

Materials

Experiments were carried out on male outbred Wistar rats 7–9 weeks old. [U-¹⁴C]Glucose and [³H]-thymidine were obtained from The Radiochemical Centre, Amersham, Bucks., U.K. The sources of all enzymes and reagents used in metabolite assays were described previously (Suter & Weidemann, 1975). Concanavalin A was initially prepared as described by Agrawal & Goldstein (1967), but for the cell-culture experiment (Fig. 1) it was obtained from Pharmacia, Uppsala, Sweden.

Horse serum was obtained from Gibco, Scotland. Eagle's medium (Dulbecco's modification) without glucose was a gift from Fr. Dr. M.-L. Lohman-Matthes and was prepared from constituents obtained from Gibco. The horse serum was dialysed for 24h against five changes of 100vol. of non-sterile phosphate-buffered saline to remove glucose.

Methods

Rat thymus lymphocytes were prepared as previously described (Culvenor & Weidemann, 1976). Cell incubations with [U-¹⁴C]glucose, and subsequent substrate and product assays for the experiments shown in Tables 1 and 3, were carried out as described by Suter & Weidemann (1975). Acetoacetate and 3-hydroxybutyrate were assayed as described by Williamson *et al.* (1962). To analyse the fate of labelled carbon derived from [U-¹⁴C]glucose (Table 2), duplicate incubations in Warburg flasks were performed for the separate assay of soluble and insoluble metabolites. After 3h incubation, HCl (0.25ml, 0.5M) was added from the side-arm. The cell suspension was homogenised with 20 strokes of a glass/glass homogenizer and made up to 4.5ml with buffer. RNA was extracted from 1.0ml of homogenate by the method of Munro *et al.* (1964), and DNA was extracted from the remaining pellet by the method of Burton (1956). The protein pellet remaining after this extraction was drained, washed with HClO₄ (3.0M) and dissolved in 98% (v/v) formic acid for liquid-scintillation counting. Lipids were extracted from 1.0ml of the original homogenate as described by Folch *et al.* (1957).

Soluble metabolites were separated on a Dowex column by the following method. Dowex 1 was mixed to a slurry with water and poured into a column of 5cm diameter. Sodium acetate (1.0M) was allowed to run through the column until no AgCl precipitate was formed when the eluate was tested with AgNO₃. [¹⁴C]Glucose, [¹⁴C]glutamate, [¹⁴C]-aspartate and [¹⁴C]lactate were separated by using the resulting Dowex (acetate form) in a column (1.0cm diam. × 15.0cm long) and a modification of the method of Busch (1953). After application of the sample

(4.5ml) the metabolites were eluted with 20ml of water (glucose), 15ml of 0.5M-acetate (glutamate), 15ml of 1.5M-acetate (aspartate) and 35ml of 3.0M-acetate (lactate). Approx. 80 fractions (1.0ml each) were collected. The fractions constituting the four radioactive peaks were pooled, rotary-evaporated and suspended in 1.5ml of buffer. Each pooled fraction was assayed enzymically for glucose, lactate, aspartate (Pfleiderer, 1965) and glutamate (Bergmeyer & Bernt, 1965). Glycogen radioactivity was determined by a modification of the diazyme method of Bartley & Dean (1968). Cell cultures of rat thymus lymphocytes were carried out as described by Wekerle (1978).

Results

Quantitative aspects of [U-¹⁴C]glucose oxidation

In the absence of added substrate, rat thymus lymphocyte suspensions consumed O₂ at a non-linear rate during the first hour of incubation and produced large quantities of NH₄⁺ (16.3 μmol/3h per

Table 1. *Effects of concanavalin A on the metabolism of [U-¹⁴C]glucose by rat thymocytes*

Rat thymus lymphocytes (1.3 × 10⁸/ml) were incubated for 3h in the presence of [U-¹⁴C]glucose (5mM, 10⁶ c.p.m./flask) ± concanavalin A (50 μg/ml) in a total volume of 3ml. Details of substrate assays and calculations are given in the text. Results for all substrates are given as μmol formed (or utilized)/3h incubation per 10¹⁰ cells ± S.E.M. for the numbers of observations (in parentheses) on separate cell preparations.

	Control (7)	+Concanavalin A (7)
O ₂ consumption	-412 ± 20	-435 ± 24
CO ₂ production	+416 ± 27	+446 ± 29
Respiratory quotient (CO ₂ /O ₂)	1.0 ± 0.02	1.03 ± 0.02
Glucose disappearance	-72.6 ± 7.3	-141.3 ± 6.9
Lactate production	+47.8 ± 3.5	+153.2 ± 9.2
Pyruvate production	+2.64 ± 0.2	+5.43 ± 0.32
10 ⁻⁴ × Radio-activity in ¹⁴ CO ₂ (c.p.m.)	4.92 ± 0.32	7.19 ± 0.57
10 ⁻³ × Sp. radio-activity of ¹⁴ CO ₂ (c.p.m./μmol)	3.36 ± 0.23	4.60 ± 0.37
Glucose to ¹⁴ CO ₂ (%)	27.7 ± 3.1	20.7 ± 1.1
Glucose to lactate (%)	37.6 ± 3.8	57.8 ± 5.3
Glucose not accounted for (%)	33.6 ± 5.4	21.5 ± 4.9
Glucose to fuel of respiration (%)	43.0 ± 3.2	57.7 ± 4.6

10¹⁰ cells). Addition of 5.0mM-glucose led to a 53% increase in the O₂ consumption, which became linear, and a total suppression of NH₄⁺ production (results not shown). Table 1 shows an analysis of the net fate of glucose carbon in thymocytes incubated with 5.0mM-[U-¹⁴C]glucose; 38% of glucose disappearance could be accounted for by lactate production, and 28% by CO₂ production, leaving 34% unaccounted for. Table 2 demonstrates that a large proportion of the glucose carbon not accounted for by lactate and CO₂ was found in glycogen, with a smaller percentage in the protein, lipid and nucleic acid pools; 14.2% of the radioactivity lost from glucose was found in glutamate and aspartate (Table 2). Suter & Weidemann (1975) have suggested that these amino acids may be labelled by isotope exchange through the glutamate and aspartate transaminase half-reactions (Haslam & Krebs, 1963; Krebs *et al.*, 1966). If ¹⁴C does enter glutamate and aspartate purely by isotope exchange, this reaction is equivalent to a loss of label potentially available for incorporation into CO₂ in the tricarboxylic acid cycle. In support of this proposal, the specific activity of recovered glutamate is identical with that of CO₂ (Table 2). The following equation was developed to calculate the percentage contribution of glucose to the fuel of thymocyte respiration. The equation compares the specific radioactivity of ¹⁴CO₂ (corrected for the loss of label to glutamate and aspartate) with the specific activity of the original glucose

(i.e. the dilution of the specific activity of glucose carbon).

Percentage contribution of glucose to respiratory fuel=

$$\frac{\text{d.p.m. in } ^{14}\text{CO}_2 + 0.142 (\text{d.p.m. lost from glucose})}{\mu\text{mol of CO}_2 \text{ produced}} \times \frac{6 (\mu\text{mol of glucose added})}{\text{d.p.m. in added glucose}} \times 100$$

On the basis of this calculation glucose contributed 43% of the respiratory fuel over 3 h of incubation (Table 1).

Acetoacetate (5.0mM) alone stimulated the basal rate of thymocyte respiration to the same extent as glucose (Table 3). The net amount of acetoacetate consumed was sufficient to account for 65% of cellular O₂ consumption when production of 3-hydroxybutyrate was subtracted. When cells were incubated with [U-¹⁴C]glucose plus acetoacetate, acetoacetate was the preferred fuel of respiration. Acetoacetate greatly inhibited the flow of glucose carbon to ¹⁴CO₂, and 90% of the net glucose uptake in its presence could be accounted for by the production of lactate and pyruvate. Thus acetoacetate also substantially decreased the percentage of glucose uptake not accounted for, which, according to the data in Table 2, may be equated with an inhibition of glycogen synthesis.

Table 2. *Effect of concanavalin A on the fate of labelled carbon from [U-¹⁴C]glucose in rat thymocytes*
The methods are given in detail in the text.

Metabolite	Control			+Concanavalin A		
	Radioactivity (d.p.m./flask)	Recovered radioactivity (d.p.m.) (excluding glucose) (%)	Specific radioactivity (d.p.m./μg- atom of C)	Radioactivity (d.p.m./flask)	Recovered radioactivity (d.p.m.) (excluding glucose) (%)	Specific radioactivity (d.p.m./μg- atom of C)
Glucose (initial)	4 633 180		51 864	4 633 180		51 864
Glucose (final)	3 756 760		48 237	3 137 260		45 280
Glucose (metabolized)	876 420			1 495 920		
Glycogen + oligo- saccharides	159 155	23.8		177 541	12.4	
Glutamate	62 669	9.3	14 882	99 308	6.9	22 537
Aspartate	32 616	4.9		41 501	2.9	
Lactate	178 179	26.5	54 297	700 470	48.9	44 540
CO ₂	183 810	27.4	13 707	312 476	21.8	18 446
RNA	10 861	1.6		19 332	1.3	
DNA	10 300	1.5		12 940	0.9	
Protein	20 468	3.1		39 149	2.7	
Lipid	12 876	1.9		25 509	2.1	
Total radioactivity (d.p.m.) recovered	4 428 094			4 569 385		

Metabolic response to concanavalin A

As Table 1 shows, addition of concanavalin A to thymocytes in the presence of 5.0 mM-[U- 14 C]glucose led to a 2-fold increase in net glucose uptake during a 3 h incubation. Despite a small but highly-reproducible stimulation of respiration, the majority of the extra glucose taken up was converted to lactate (75%). The lactate/pyruvate ratio, which may be taken as index of the redox state of the cytoplasmic nicotinamide nucleotides, was increased by 50% in the presence of concanavalin A. The net appearance of label in CO_2 , and the specific activity of the CO_2 produced, increased by 30–40%, leading to an increase in the estimate of the contribution of glucose to the fuel of respiration (which was calculated by using the appropriate correction for the smaller percentage of glucose carbon flowing to glutamate and aspartate, Table 2). The percentage of glucose uptake accounted for by macromolecule synthesis (protein, RNA and lipids) was not significantly altered in the stimulated cells, and a doubling in the recovery of label in these fractions paralleled the doubling in the rate of glucose uptake (Table 2). At this early stage of transformation, little specific stimulation of the incorporation of glucose carbon into DNA was observed.

Relatively little effect of concanavalin A on the flow of label into the glycogen pool was observed (Table 2). This result may be criticized on two grounds. Firstly, the cells were prepared in glucose-free media and may have been depleted of glycogen before incubation. Secondly, since net glycogen was estimated by difference (Bartley & Dean, 1968), the method itself has inherent inaccuracies.

The addition of acetoacetate (Table 3) did not affect the glycolytic stimulation induced by concanavalin A, but a much larger proportion of the glucose taken up was converted into lactate. $^{14}\text{CO}_2$ production, as observed in unstimulated cells, was strongly inhibited by acetoacetate, but concanavalin A was still able to stimulate this process. Concanavalin A had no effect on acetoacetate oxidation.

Effect of glucose concentration in the medium on [^3H]thymidine uptake in concanavalin-stimulated cell cultures

In the absence of glucose or concanavalin A, [^3H]thymidine incorporation was almost undetectable in thymocyte cultures. Even in the presence of 5 mM-glucose incorporation was less than 500 c.p.m. Fig. 1 shows that [^3H]thymidine incorporation in the presence of concanavalin A responded to the concentration of glucose in the medium in a biphasic manner. The estimated glucose concentration in the culture medium was less than 5 μM . Even at this concentration, concanavalin A caused a marked stimulation of [^3H]thymidine incorporation. Addition of glucose at concentrations as low as 25 μM caused a

Fig. 1. Effect of glucose concentration in the medium on [^3H]thymidine uptake in concanavalin A-stimulated rat thymocyte cultures

Cells (5×10^6) were cultured in a medium containing Eagle's medium (glucose-free), 15% (v/v) horse serum (dialysed), 2-mercaptoethanol (0.05 mM), concanavalin A (10 $\mu\text{g}/\text{ml}$) and appropriate additions. After 30 h [^3H]thymidine (1 μCi , 10 μM) was added to each well and the cells were harvested 16 h later. The result shown is a representative of four separate experiments performed; each point is the average for three determinations. ○, control; □, +5 mM-lactate; ●, +2.5 mM-inosine.

doubling in the incorporation of [^3H]thymidine. No further response to glucose occurred until the medium glucose concentration was raised above 1.0 mM, when a further doubling occurred. Neither added lactate (Fig. 1) nor 3-hydroxybutyrate (results not shown) altered the pattern of the response to glucose. In contrast, inosine substantially decreased the response to added glucose (Fig. 1), although a small increase in [^3H]thymidine uptake was still observed at glucose concentrations above 1.0 mM.

Discussion

The ability of thymocytes to transform when glucose is present in the medium at very low concentrations (Fig. 1) suggests that enhanced glucose metabolism as such is not essential for the initiation of DNA synthesis in response to concanavalin A. The K_m of the glucose carrier for glucose (0.3 mM; Yasmeen *et al.*, 1977) is not altered by concanavalin A (Yasmeen *et al.*, 1977), and thus the rate of glucose uptake at the low glucose concentrations reported in Fig. 1 is lower than that observed in unstimulated cells at saturating glucose concentrations. Naiditch & Cunningham (1977) used a similar experimental approach to reach the same conclusion with respect

to the control of fibroblast proliferation. Nevertheless, Fig. 1 demonstrates that the continued presence of saturating glucose concentrations is necessary to sustain maximal rates of DNA synthesis. Thus, the rate of DNA synthesis in the presence of concanavalin A may be permitted to double as a result of an increase in the rate of glucose uptake. The data in Table 2 demonstrate that concanavalin A stimulates the flow of glucose carbon into the RNA pool, and that this increase is proportional to the increase in the rate of glucose uptake. Bissell (1976) has suggested that the activity of the oxidative segment of the pentose phosphate pathway in virus-transformed cells is proportional to the rate of glucose transport. Hovi *et al.* (1976) and Chambers *et al.* (1974) have observed a stimulation of purine-base biosynthesis in mitogen-treated lymphocytes. Inhibitors of the synthesis *de novo* of purine bases inhibit lymphocyte proliferation, suggesting that the activity of the salvage pathway of purine synthesis is insufficient to support DNA synthesis. Hovi *et al.* (1975) have reported a transient increase in the concentration of phosphoribosyl pyrophosphate in phytohaemagglutinin-treated human peripheral blood lymphocytes, which reaches a peak after 30 min and then slowly returns to basal values. The concentration of phosphoribosyl pyrophosphate may be an important factor in the regulation of purine biosynthesis (Green & Martin, 1974). Additionally, increased intracellular concentrations of glucose 6-phosphate (Culvenor & Weidemann, 1976; Keig, 1973) may be necessary for maximal purine biosynthesis from glucose in mitogen-treated lymphocytes, and may be the direct cause of the increase in phosphoribosyl pyrophosphate observed by Hovi *et al.* (1975). Since the maximum catalytic activity of phosphofructokinase in lymphoid tissue is much higher than that of the glucose carrier (Yasmeen *et al.*, 1977), an elevation of the steady state concentration of intracellular glucose 6-phosphate would require a very large stimulation of glucose transport and/or inhibition of phosphofructokinase. The actual change in glucose uptake in individual cells responding to concanavalin A is probably very much larger than the doubling observed in Table 1. Reeves (1977), Whitesell *et al.* (1977) and Yasmeen *et al.* (1977) have observed that rat thymocyte suspensions consist of two populations of cells, distinguishable on the basis of their kinetics of glucose transport. The 'fast-transporting' cells have been identified as lymphoblasts (Reeves, 1977), larger mature T-lymphocytes that can undergo cell division at a rapid rate (Whitfield *et al.*, 1976). Whitesell *et al.* (1977) have shown, by using direct kinetic measurements and radioautography, that stimulation of glucose transport by concanavalin A involves the conversion of 'slow-transporting' into 'fast-transporting' cells, which exhibit a 20–40-fold higher rate of glucose

transport. The magnitude of this increase is masked in our experiments by the pre-existence of 20% of the cells in the 'fast-transporting' state (Reeves, 1977).

Kay (1976) has observed that DNA synthesis in mitogen-treated lymphocytes is glucose-dependent, with no synthesis occurring in the absence of added glucose. Our failure to observe complete suppression of DNA synthesis in the absence of glucose may have been due to the incomplete removal of glucose from the horse serum. Kay (1976) also observed a substantial reduction in phytohaemagglutinin-stimulated RNA and protein synthesis in the absence of added glucose. This observation may also reflect a direct requirement for glucose carbon, as evidenced by the concanavalin A-induced increase in the flow of glucose carbon into these pools (Table 2).

Enhanced glucose metabolism in mitogen-treated lymphocytes is probably transient. Resch *et al.* (1976) have shown that lymphocytes escape the inhibitory effect of cytochalasin B, an inhibitor of thymocyte glucose transport, only a few hours after the addition of concanavalin A. We have demonstrated that the inhibitory effects of cytochalasin lymphocyte transformation can be explained solely in terms of its effects on glucose transport (Hume *et al.*, 1978a). Wang *et al.* (1976) have observed a large peak of lactate production associated with maximal DNA synthesis, suggesting that glycolysis may be reactivated at this stage. The experiments of Toyoshima *et al.* (1976) suggest that there are, indeed, two stimulatory events in lymphocyte transformation.

The increase in the percentage contribution of glucose to the fuel of respiration in concanavalin A-treated cells (Table 1) may be an important device to spare precursors for macromolecule synthesis. Concanavalin A does not affect acetoacetate oxidation and hence probably does not directly stimulate the oxidation of acetyl-coenzyme A by the tricarboxylic acid cycle. The activity of the pentose phosphate pathway, although pertinent to the above discussion, is rather low in lymphoid tissue (Hedekov, 1968; Suter & Weidemann, 1975) and is probably insufficient to account for a specific increase in $^{14}\text{CO}_2$ production from $[\text{U-}^{14}\text{C}]\text{glucose}$. We have confirmed (C. Bryant, unpublished work), by using specifically labelled glucose, that increased $^{14}\text{CO}_2$ production in response to concanavalin A (Table 1) is due to a specific increase in pyruvate oxidation (see Hume *et al.*, 1978b).

The effect of acetoacetate on thymocyte glycolysis (Table 3) differs markedly from that observed in brain and muscle, where ketone-body oxidation suppresses glycolysis (Newsholme *et al.*, 1977). Glycolytic inhibition by fatty acids or ketone bodies is believed to be the result of an inhibition of phosphofructokinase by elevated tissue concentrations of citrate. Although lymphoid-tissue phosphofructokinase is extremely sensitive to inhibition by citrate

Table 3. *Effect of acetoacetate on [U-¹⁴C]glucose metabolism and the response to concanavalin A in rat thymocytes*
 Details of the method are given in the text and in Table 1. Results are given as $\mu\text{mol/3 h}$ per 10^{10} cells $\pm$ S.E.M. for three experiments. Glucose and acetoacetate were each added at a final concentration of 5 mM.

Substrate added $\pm$ Concanavalin A (50 $\mu\text{g/ml}$)	Acetoacetate		Glucose		Glucose+acetoacetate	
	—	+	—	+	—	+
Oxygen uptake	391 $\pm$ 18	400 $\pm$ 22	360 $\pm$ 12	391 $\pm$ 12	414 $\pm$ 20	421 $\pm$ 25
Glucose uptake	—	—	55 $\pm$ 4	122 $\pm$ 4	45 $\pm$ 1	105 $\pm$ 7
Lactate production	—	—	27 $\pm$ 3	134 $\pm$ 5	73 $\pm$ 3	172 $\pm$ 4
Pyruvate production	—	—	1.3 $\pm$ 0.2	4.5 $\pm$ 0.2	8.7 $\pm$ 0.6	15.9 $\pm$ 0.9
Acetoacetate uptake	84 $\pm$ 8	81 $\pm$ 7	—	—	91 $\pm$ 13	81 $\pm$ 14
β -Hydroxybutyrate production	20 $\pm$ 3	19 $\pm$ 1	—	—	26 $\pm$ 1	25 $\pm$ 1
$10^{-4} \times$ Radioactivity (c.p.m.) in ¹⁴ CO ₂	—	—	6.3	10.0	1.5	2.8

(Weidemann *et al.*, 1977), acetoacetate oxidation causes only a 20% suppression of glycolysis (Table 3). This observation is consistent with glucose transport, rather than phosphofructokinase, being the rate-limiting step for thymocyte glycolysis (Yasmeen *et al.*, 1977).

The inhibitory response of [³H]thymidine incorporation to inosine observed in Fig. 1 was surprising. Nordeen & Young (1977) have shown that inosine is adequately metabolized by thymocytes, and unlike adenosine, does not cause cyclic adenosine monophosphate accumulation. Since inosine breakdown yields ribose, we consider that inosine should, to some extent, replace glucose. Inosine does not affect glucose transport in the presence or absence of concanavalin A (D. A. Hume, unpublished work), and hence probably acts by inhibiting one of the enzymes of purine biosynthesis (Snyder *et al.*, 1976).

The results presented here indicate that enhanced aerobic glycolysis is a normal function of mitogenically-transformed cells, and is not associated specifically with malignancy. Lactate production *per se* is not a significant function of stimulated glycolysis, since the addition of lactate does not alter the requirement for glucose in transformation (Fig. 1). The principal function of enhanced aerobic glycolysis may be to raise the concentrations of glycolytic intermediates (Culvenor & Weidemann, 1976) and thus stimulate the pathways of macromolecule synthesis, or permit their stimulation by other signals. Secondary control of the glycolytic pathway at the level of phosphofructokinase may regulate the amounts of glucose 6-phosphate available for nucleic acid synthesis, whilst control at the level of pyruvate kinase (Culvenor & Weidemann, 1976) may regulate triose phosphate concentrations for triacylglycerol synthesis. Our results have shown that the increase in the flow of glucose carbon into all the macromolecule pools in response to concanavalin A is proportional to the increase in the rate of glucose uptake.

Thus glucose transport may be significant in the regulation of macromolecule synthesis. If, as proposed, glucose metabolism is transiently increased in mitogenically transformed lymphocytes, feedback control mechanisms may exist for switching-off the glucose carrier, and the absence of these mechanisms may represent a potential defect in malignantly transformed cells.

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