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# Glucose contribution to nucleic acid base synthesis in proliferating hepatoma cells: a glycine-biosynthesis-mediated pathway

Hervé BISMUT,* Martine CARON,† Colette COUDRAY-LUCAS† and Jacqueline CAPEAU†‡

*Laboratoire de Biologie, U.E.R. d'Odontologie, Université Paris 7, and †Laboratoire de Biologie Cellulaire, INSERM U402, Faculté de Médecine Saint-Antoine, 75571 Paris Cédex 12, France

The coupling of glycolysis to serine and glycine metabolism was studied in fast-growing Zajdela hepatoma cultured cells. During the exponential phase of growth, occurring between 12 and 72 h, cells exhibited a decreased glycogen content together with a high glycolytic activity. Glycogen labelling, evaluated by 1 h-pulse experiments with [U-$^{14}$C]glucose (5.5 mM), was minimal during the first 48 h and increased 2.5-fold at 72 h and 8-fold at 96 h, at which times it was also stimulated 2-fold by 10 nM insulin. [U-$^{14}$C]Glucose carbons were incorporated into nucleic acid bases, with maximal incorporation at 72 h, the rate of nucleotide base labelling exceeding that of glycogen during the first 2 days of culture. Incubation of the cells with [U-$^{14}$C]glucose resulted in the release into the medium of $^{14}$C-labelled glycine, the first intermediate formed on the route from serine to DNA. The rate of release per cell decreased as a function of cell growth, concomitantly with an increased rate of glucose carbon incorporation into nucleotide bases. The latter implied the intermediary formation of amino acids since the transaminase inhibitor cycloserine (10 mM), which totally inhibited [$^{14}$C]glycine release, decreased by 65% nucleotide labelling from [U-$^{14}$C]glucose. A dose-dependent inhibition by serine of the rate of [U-$^{14}$C]glucose carbon incorporation into nucleotide bases was observed, which was maximal at 5 mM serine. These metabolic flux measurements indicate that glucose can be used as a precursor of nucleic acid synthesis. These results strongly suggest that this process is to a large extent mediated by a serine/glycine-biosynthesis-mediated pathway, and reinforce the hypothesis that glycolysis contributes to enhancing the provision of precursors required for cell proliferation.

## INTRODUCTION

In liver, the main fate of glucose taken up from the blood following a carbohydrate-rich diet is storage as glycogen or catabolism through glycolysis and, under aerobic conditions, oxidative metabolism. The glycolytic pathway is linked to serine metabolism, since 3-phosphoglycerate can be channelled to the 'phosphorylated' pathway of serine biosynthesis and since the amino acid can be converted into 2-phosphoglycerate via an analogous 'non-phosphorylated' pathway initiated by serine aminotransferase [1]. In addition to its contribution to protein and phospholipid syntheses, serine plays a unique role in the biosynthesis of DNA as a major one-carbon donor for thymidylate synthesis via the formation of 5,10-methylenetetrahydrofolate. This transfer of a one-carbon unit from serine to tetrahydrofolate, which is catalysed by serine hydroxymethyltransferase, results in the formation of glycine which takes part in the assembly of nucleotide purine rings (reviewed in [2]).

While the physiological significance of the serine cycle in normal adult hepatocytes remains questionable, the activity of the enzymes involved in the phosphorylated pathway of serine biosynthesis has been reported to be increased in late-fetal rat liver when compared with the adult tissue [3–5]. Accordingly, in cultured fetal rat hepatocytes, glucose taken up was shown to be simultaneously diverted towards the pathways of glycogen formation and of serine biosynthesis [6,7]. On the other hand, the assessment of enzyme activities in various hepatoma cells reported by Snell and co-workers indicates that the biosynthesis of serine from glycolysis is metabolically coupled to the use of serine for the formation of nucleotide precursors [2,8–10]. These studies therefore suggest that the diversion of glucose-derived intermediates towards serine biosynthesis and ultimately nucleotide and protein formation might be responsible for the growth advantages of both developing and proliferating cells over normal adult tissues. However, although glucose is undoubtedly converted into nucleic acid bases in such growing liver-derived cells, no direct flux measurements have as yet been published.

The aim of the present paper was to evaluate the capacity of hepatoma cells to incorporate glucose carbons into nucleic acids via a serine-biosynthesis-mediated pathway. Glucose carbon incorporation into glycogen and nucleic acids was determined in cultured Zajdela rat hepatoma cells. These are rapidly growing cells that incorporate glucose into glycogen [11–13], a function generally lost in hepatic tumour cells. The flux measurements reported herein revealed that during the exponential phase of cell growth glucose diversion towards the biosynthesis of nucleic acid bases predominates over its incorporation into glycogen. The results indicate that glucose can be used as a precursor of nucleotide base formation through a transaminase-dependent pathway mediated by serine and glycine formation, and support the hypothesis that glucose-derived intermediates contribute to enhancing the provision of precursors required for cell proliferation.

## MATERIALS AND METHODS

### Materials

Dulbecco's modified Eagle's medium (DMEM) and donor calf serum were from Gibco. L-Serine and DL-cycloserine were purchased from Sigma. Insulin (monocomponent porcine insulin, 25 units/mg) was obtained from Novo Laboratories. D-[U-$^{14}$C]Glucose and D-[1-$^{14}$C]glucose were from New England Nuclear, and L-[U-$^{14}$C]serine, L-[U-$^{14}$C]leucine and [*Me*-$^{3}$H]thymidine were from Amersham. Other biochemicals (Merck) were of the best grade available.

Abbreviation used: DMEM, Dulbecco's modified Eagle's medium.

‡ To whom correspondence should be addressed.

### Cell culture

Cells were obtained from the heterogeneous ZHC cell population by subcloning ZD 9 strain, which was selected for its high insulin receptor and glycogen contents. The cells were grown in DMEM containing 44 mM glucose, 5% (v/v) newborn-calf serum and antibiotics (100 units/ml penicillin and 1 mg/ml streptomycin). Cells were cultured in 75 $cm^2$ flasks at 37 °C in a humidified 95% $O_2$/5% $CO_2$ atmosphere. Confluent cells were replated in 12-well plates at a density of $0.2 \times 10^6$ cells/well and cultured for 5 days in DMEM containing 5.5 mM glucose and 5% (v/v) donor calf serum, the medium being replaced each 24 h. Periods of culture longer than 96 h resulted in cell detachment, which made metabolic studies difficult to interpret. When [*Me*-$^3$H]thymidine (0.5 mCi/ml) was used as a precursor of DNA during 1 h-pulse experiments, label incorporation into trichloroacetic acid-precipitable material progressively increased between 12 and 96 h, correlatively to the cell number and the protein content. This rate tended to decrease thereafter despite the increase in cell number (results not shown). For that reason, labelling experiments using [U-$^{14}$C]glucose were performed up to 96 h.

### Labelling experiments

In short-term (1–4 h) labelling experiments, the medium was replaced with 0.5 ml of serum-free DMEM containing 5.5 mM glucose. After 15 min at 37 °C, the medium was replaced with 0.5 ml of a similar fresh medium supplemented with the labelled precursors and the agents to be tested. After 1 or 4 h of incubation at 37 °C, the cells were washed three times with 1 ml of ice-cold phosphate-buffered saline (PBS) and stored at −80 °C until required for use. In 24 h-labelling experiments, the medium was replaced with 2 ml of a fresh serum-supplemented medium containing 5.5 mM [U-$^{14}$C]glucose. After 24 h, aliquots of medium were collected and the cultures were stopped as described above.

### Labelled glycogen and nucleotide determination

Cells were treated with 0.5 ml of 1 M NaOH for 2 h at 20 °C to promote cell solubilization, after which time the wells were rinsed with 0.5 ml of water. Aliquots (0.4 ml) of the cell extract were used for measurements of glycogen content and glycogen labelling as described by Plas et al. [14]. Aliquots (0.4 ml) of the cell extract were supplemented with 0.6 ml of water and used for nucleotide precipitation in 1 M $HClO_4$ at 4 °C as described in [10], by using BSA (10 mg/ml) as a carrier. Purine and pyrimidine bases were liberated from the nucleic acid extract by hydrolysis at 100 °C and centrifugation [15]. The absence of detectable glycogen or protein residues in the supernatant was checked by spectrophotometry using the anthrone [16] and the Coomassie Blue dye binding [17] methods respectively. Radioactivity incorporated into glycogen and nucleotide bases was determined by liquid-scintillation counting. Cell protein content was evaluated according to Bradford [17].

### Labelled protein and nucleic acid determination

The incorporation of [U-$^{14}$C]glucose into both proteins and nucleic acids, of [U-$^{14}$C]leucine into proteins and of [*Me*-$^3$H]thymidine into DNA were determined by counting trichloroacetic acid-precipitable radioactivity [18]. Briefly, cells were treated with 1 ml of 5% trichloroacetic acid for successive periods of 30 min and 10 min at 4 °C. The trichloroacetic acid precipitate was rinsed with 1 ml of ice-cold PBS and then extracted by successive additions of 0.5 ml of 1 M NaOH (2 h at 20 °C) and 0.5 ml of water. Radioactivity incorporated was determined by liquid-scintillation counting. Trichloroacetic acid-precipitable proteins represented about 80% of total cellular protein content.

### Determination of metabolites

Labelled metabolites present in the media were separated, identified and measured by TLC as previously described [7]. Briefly, samples of medium were applied on silica gel G plates (Merck) and migration was performed in propan-2-ol/water (7:3, v/v). The plates were dried, autoradiographed, and the radioactivity corresponding to labelled areas co-migrating with authentic standards was determined by liquid scintillation. Glucose concentration in the medium was determined by the glucose oxidase procedure (Beckman Astra 8). Lactate concentration was measured enzymically (Boehringer-Mannheim test-combination) according to Hohorst [19]. Amino acid concentrations were determined by ion-exchange chromatography (Beckman 6300) after deproteinization with sulphosalicylic acid (50 mg/ml).

### Expression and statistical analysis of results

Label incorporation is expressed in nmol of glucosyl units per mg of total or trichloroacetic acid-precipitable cell protein. Production or consumption of labelled substrates during 24 h-labelling experiments is expressed in nmol of glucosyl units divided by the mean value of protein content during the same 24 h period. Data are presented as means ± S.E.M. for the number of independent experiments indicated in the legends of the Figures. Student's *t* test for paired samples was used for the statistical analysis of the results.

## RESULTS

### Characteristics of cell growth as a function of the time of culture

The growth characteristics of confluent ZHC cells replated at a low density ($0.2 \times 10^6$ cells/well) were estimated over 5 days of culture. Cell multiplication paralleled the cell protein content for up to 96 h, after which time the protein content remained constant although the cell number still increased (results not shown). The cells exhibited an exponential phase of growth between 12 and 72 h of culture, as indicated by the time-dependent cell protein content determined over 96 h (Figure 1a). The various parameters of glucose metabolism were then studied as a function of protein content, taken as an index of cell multiplication for up to 4 days of culture.

### Lactate production and glycogen content as a function of cell growth

The release of lactate into the medium reflected the high glycolytic activity of the cells, which appeared to be correlated with cell number and glucose consumption (Figure 1b). Figure 1(c) shows that cells contained high amounts of glycogen 6 h after replating (first time point on the curve), corresponding to about 500 $\mu$g/mg of protein. The glycogen content per cell markedly decreased during the first 48 h while the cell glycogen concentration remained stable in the slow-growing phase. The fact that lactate production per well slightly predominated over the net glucose consumption suggests that lactate originated from other precursors, most likely amino acids.

### Glycogen and nucleic acid formation from $^{14}$C-labelled glucose

Pulse experiments were performed at different times after plating by using [U-$^{14}$C]glucose at a final concentration of 5.5 mM. The

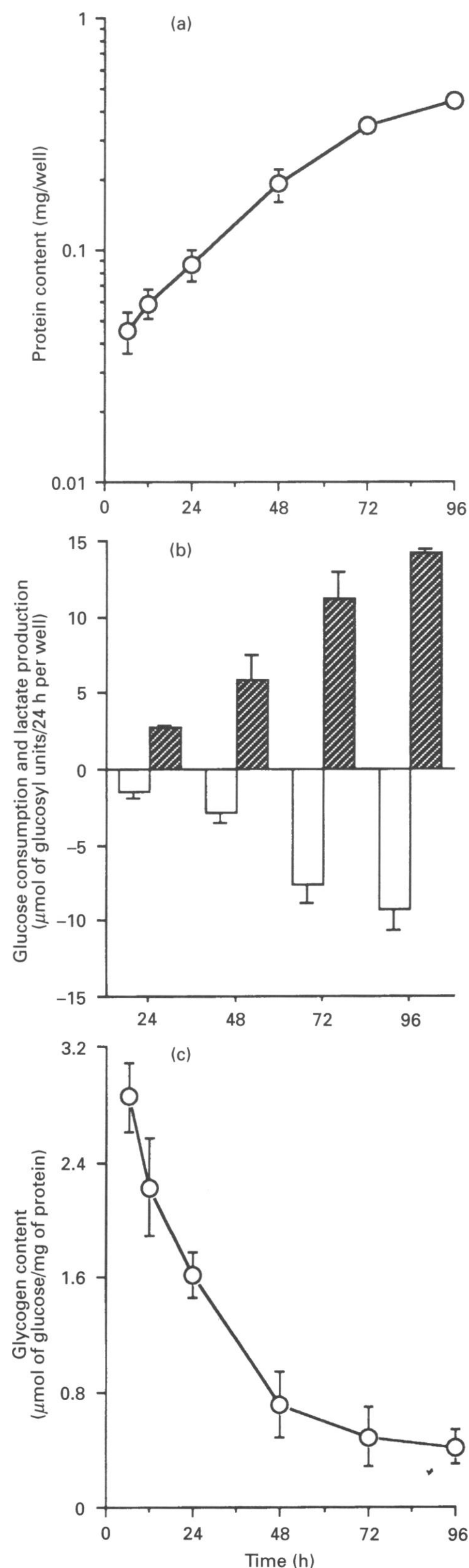


**Figure 1 Cell protein content, glucose consumption, lactate production and cell glycogen content as a function of the time of culture**

At the times of culture indicated, cell protein content (**a**), the variation in glucose (open bars) and lactate (hatched bars) concentrations in the medium (**b**), and cell glycogen content (**c**) were determined. Results are presented as means ± S.E.M. for four to five independent experiments performed with different cell preparations.

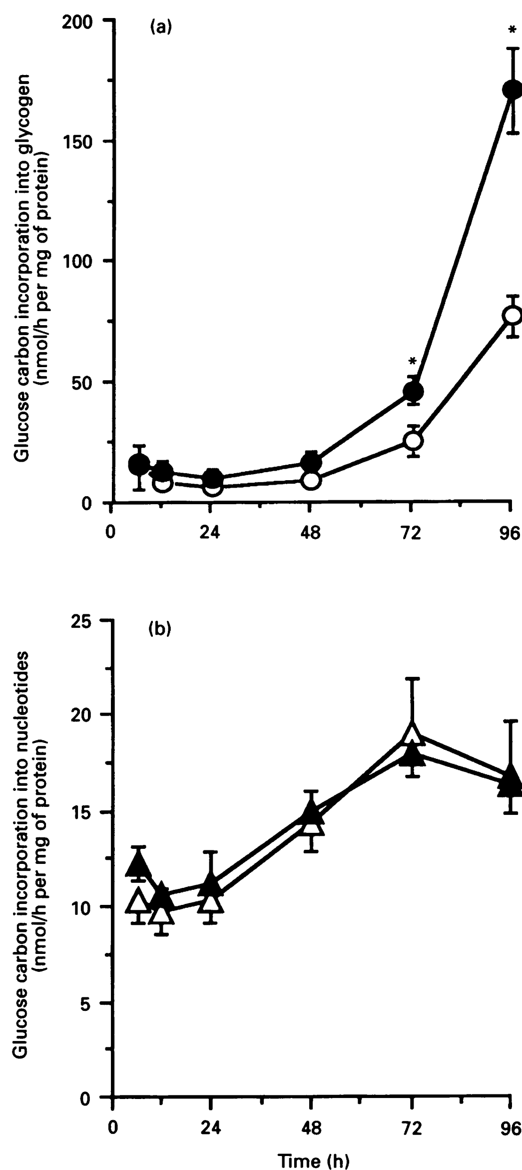


**Figure 2 [U-$^{14}$C]Glucose incorporation into glycogen and nucleotide bases as a function of the time of culture**

At the times of culture indicated, the medium was removed and replaced with 0.5 ml of a fresh serum-free medium containing 5.5 mM [U-$^{14}$C]glucose (0.36 $\mu$Ci/$\mu$mol) and supplemented (●, ▲) or not (○, △) with 10 nM insulin. Radioactivity incorporated into glycogen (**a**) and nucleotide bases (**b**) was measured 1 h later. Results are presented as means ± S.E.M. for three independent experiments performed with different cell preparations. *$P < 0.01$ compared with the corresponding control in the absence of insulin.

rate of label incorporation into glycogen did not vary between 6 and 48 h, and represented about 15 nmol of glucose/h per mg of protein (Figure 2a). By contrast, [U-$^{14}$C]glucose carbon incorporation was markedly increased at 72 and 96 h since it represented 2.5- and 8-fold, respectively, that determined during the first 2 days. This high glycogenic activity was concomitant with glycogenolysis, since glycogen content per cell did not vary after 48 h (Figure 1c). To characterize glucose metabolism further, the fate of [U-$^{14}$C]glucose was studied in the presence of insulin as a glycogenic agent. As shown in Figure 2(a), insulin (10 nM) did not significantly modify glycogen labelling during

**Table 1 Production of labelled glycine and serine from [U-$^{14}$C]glucose as a function of the time of culture**

Confluent cells were replated in 2 ml of medium containing 5.5 mM [U-$^{14}$C]glucose (0.136 $\mu$Ci/$\mu$mol). The conditioned medium was replaced each 24 h with a fresh one containing 5.5 mM [U-$^{14}$C]glucose (0.136 $\mu$Ci/$\mu$mol). Samples of medium were collected at the times of culture indicated and the variation in serine and glycine concentrations as well as the release of labelled serine + glycine were determined. Results are presented as means ± S.E.M. for three independent experiments performed with different cell preparations. * $P < 0.05$ and ** $P < 0.01$ compared with concentration in the fresh medium.

| | Serine and glycine concn. in the medium ($\mu$M) | | Serine and glycine release (nmol of glucosyl units/24 h per mg of protein) | | |
|---|---|---|---|---|---|
| Time (h) | Serine | Glycine | Serine | Glycine | [$^{14}$C](Glycine + serine) |
| 0 | 377 ± 3 | 384 ± 7 | | | |
| 24 | 378 ± 6 | 416 ± 7** | 3 ± 6 | 270 ± 21 | 923 ± 138 |
| 48 | 357 ± 9 | 424 ± 6* | −100 ± 90 | 200 ± 74 | 347 ± 74 |
| 72 | 325 ± 8** | 429 ± 10* | −197 ± 24 | 117 ± 35 | 270 ± 76 |
| 96 | 340 ± 14* | 458 ± 24* | −55 ± 24 | 123 ± 38 | 250 ± 40 |

the first 48 h of culture. However, a 2-fold stimulation was observed at 72 and 96 h, the times when the basal glucose carbon incorporation into glycogen had dramatically increased.

The high glycolytic activity revealed in Figure 1 suggested that glucose taken up by the cells was directed towards metabolic pathways other than glycogenesis. Pulse experiments performed after 48 h of culture showed that 29 ± 2 nmol of glucosyl units/h per mg of protein ($n = 4$) were incorporated into trichloroacetic acid-precipitable material, including proteins and nucleic acids, which represents twice as much as the incorporation into glycogen.

[U-$^{14}$C]Glucose carbon incorporation into nucleic acid bases was assessed by measuring the radioactivity after hydrolysis of purine and pyrimidine bases from total nucleic acids. The rate of $^{14}$C incorporation increased between 12 and 72 h, at which time it was maximal and corresponded to about 18 nmol of glucosyl units/h per mg of protein, whether insulin was present or not (Figure 2b). In order to determine to what extent nucleotide base labelling could have been overestimated by the presence in the hydrolysate of [$^{14}$C]ribose- or [$^{14}$C]deoxyribose-containing mono- or dinucleotides [20], cells were incubated in the presence of [1-$^{14}$C]glucose, the radioactivity of which is eliminated during the conversion of glucose 6-phosphate into ribulose 5-phosphate, the precursor of ribose 5-phosphate through the pentose-phosphate pathway [21]. Under these conditions, label incorporation into nucleotides, which mostly results from $^{14}$C incorporation into DNA thymine and also into DNA and RNA purines [22], was found to represent 85–95 % of that measured with [U-$^{14}$C]glucose, whatever the period of the culture considered (results not shown). This indicates that the radioactivity due to labelled ribose and deoxyribose units in the hydrolysate was minimal when compared with that due to labelled bases. Thus, these results show that glucose utilization for nucleic acid bases predominated over glucose diversion towards glycogen synthesis during the first 2 days of culture, the latter pathway becoming predominant and insulin-sensitive when the cell growth slowed down.

## Conversion of [U-$^{14}$C]glucose into [$^{14}$C]serine and [$^{14}$C]glycine

The postulated conversion of glucose into serine prior to incorporation into nucleic acids implies the biosynthesis *de novo* of serine and also of glycine, the first intermediate on the route from serine to nucleotides. The variations of amino acid concentrations in the medium were characterized by a net production of glycine and a net consumption of serine (Table 1), while the concentration of most other amino acids, except alanine which was absent from the fresh medium, reflected a net uptake (results not shown). The high rate of consumption of glutamine, and to a lesser extent of leucine, isoleucine and valine, suggests that these compounds contributed to lactate synthesis. When the flux of amino acids between the cells and the medium was expressed per mg of cell protein, a higher glycine release was shown in the first 2 days of culture, while serine consumption peaked between 24 and 72 h (Table 1).

To estimate the contribution of glycolysis to glycine production, the cells were cultured in the presence of [U-$^{14}$C]glucose and the medium analysed each 24 h. The $^{14}$C incorporation into glycine (Table 1) indicated that [U-$^{14}$C]glucose-derived metabolites contributed to the biosynthesis *de novo* of glycine. Time-dependent labelled glycine release expressed per mg of protein suggested that the level of the intracellular accumulation of glucose-derived glycine, which results from the equilibrium between glycine biosynthesis and its utilization, was higher in the early phase of the culture and tended to stabilize when glucose carbon incorporation into nucleotide bases proceeded at its maximal rate (Figure 2b). The apparent underestimation of the net increase in glycine concentration when compared with labelled glycine release was probably due to a concomitant reverse flux of the amino acid from the medium to the cell. It is also possible that the co-migration of labelled serine and glycine in the TLC solvent system used could have led to a slight overestimation of labelled glycine production, which fact would not alter the interpretation of the data since the only known route from glucose to glycine is mediated by serine formation [23]. Thus the present results reveal that the biosynthesis *de novo* of glycine plus serine from glucose-derived metabolites is operative in ZHC cells.

## Effects of cycloserine and serine on [U-$^{14}$C]glucose carbon incorporation into nucleotides

The diversion of glucose-derived substrates to serine and glycine prior to incorporation into nucleic acids actually requires the phosphoserine aminotransferase-catalysed transamination step by which phosphohydroxypyruvate is converted into phosphoserine [1]. The action of cycloserine, an inhibitor of cytosolic transaminases [24], was tested after 48 h of culture. At first, we checked its inhibitory effect on this step. Figure 3 shows the dose-dependent effect of cycloserine on labelled glycine (+ serine) release after a 4-h incubation in the presence of [U-$^{14}$C]glucose.

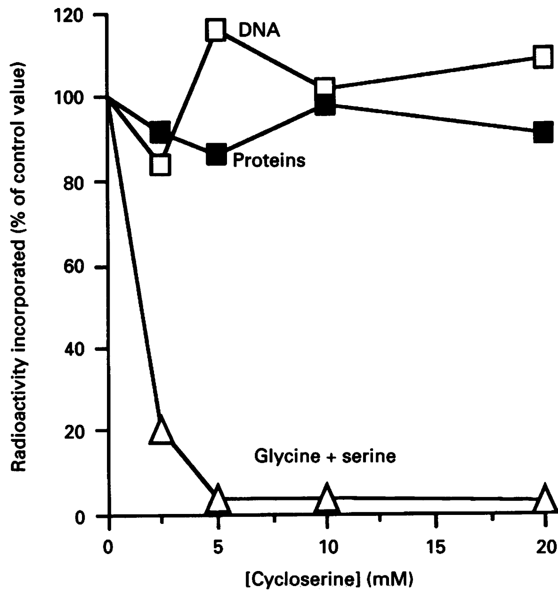


**Figure 3 Effect of cycloserine on glucose conversion into glycine and serine, on leucine incorporation into proteins and on thymidine incorporation into DNA**

After 48 h, the medium was removed and replaced with 0.5 ml of a fresh serum-free medium containing 5.5 mM [U-$^{14}$C]glucose (3.6 $\mu$Ci/$\mu$mol), 0.8 mM [U-$^{14}$C]leucine (0.2 $\mu$Ci/$\mu$mol) or $0.08 \times 10^{-3}$ mM [*Me*-$^{3}$H]thymidine (25 $\mu$Ci/$\mu$mol) and supplemented with cycloserine at the concentrations indicated. After 4 h, samples of medium were collected for the determination of labelled serine + glycine and the cultures were stopped. Radioactivity incorporated into glycine + serine (△) and into cellular trichloroacetic acid-precipitable proteins (■) and DNA (□) was determined. A representative experiment is shown where the S.D. values of measurements of triplicate cultures were in all cases less than 15% of the mean values shown. The control values in the absence of cycloserine for labelled (glycine + serine), proteins and DNA were $72 \pm 9$ nmol of glucosyl units/mg of protein, $281 \pm 36$ nmol of leucine/mg of protein and $0.12 \pm 0.02$ nmol of thymidine/mg of protein respectively.

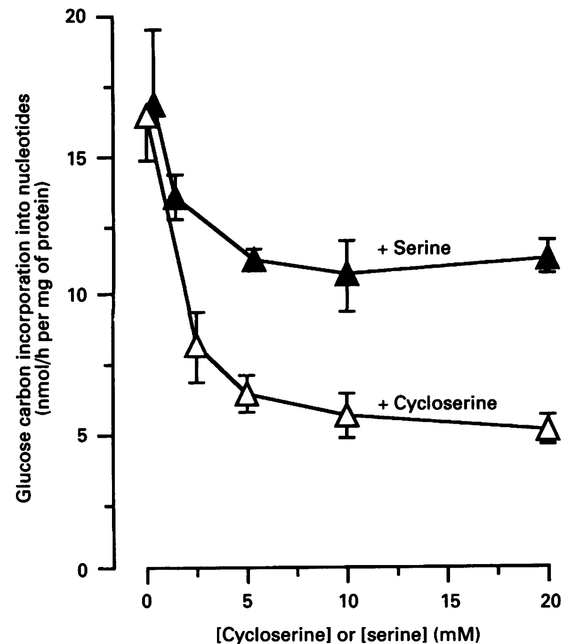


**Figure 4 [U-$^{14}$C]Glucose incorporation into nucleotide bases as a function of the concentration of cycloserine or serine**

After 48 h, the medium was removed and replaced with 0.5 ml of a fresh serum-free medium containing 5.5 mM [U-$^{14}$C]glucose (0.36 $\mu$Ci/$\mu$mol) and supplemented with cycloserine (△) or serine (▲) at the concentrations indicated. Serine concentration in the control medium was 0.4 mM. Radioactivity incorporated into nucleotide bases 1 h later is presented as means ± S.E.M. for three independent experiments performed with different cell preparations.

A total inhibition was observed with 5 mM cycloserine, indicating that this agent actually strongly inhibits serine and glycine biosynthesis from glycolysis. Under similar conditions [$^{14}$C]leucine and [$^{3}$H]thymidine incorporations into proteins and DNA were not affected, which rules out possible toxic effects of cycloserine on protein synthesis and on cell growth. As shown in Figure 4, a dose-dependent inhibition of [U-$^{14}$C]glucose carbon incorporation into nucleotide bases was observed, with a maximal inhibition of 70% obtained at 10 mM. At this concentration, cycloserine did not alter glycogen labelling [$18.7 \pm 0.1$ and $18.9 \pm 0.6$ nmol/h per mg of protein ($n = 3$) in the presence and absence, respectively, of 10 mM cycloserine], which is in favour of a selective effect of cycloserine on transamination steps.

To characterize further the pathways from glucose to nucleic acid bases, pulse experiments using [U-$^{14}$C]glucose were performed in the presence of increasing concentrations of L-serine in the medium. Indeed, this amino acid is expected to alter the pathway from glucose to nucleotides mediated by serine formation, since it acts as an inhibitor of phosphoserine dephosphorylation, the last step of the 'phosphorylated pathway' of serine biosynthesis [25]. Nucleotide base labelling was altered when the serine concentration in the medium was increased. A maximal effect was observed in the presence of 5 mM serine (Figure 4), label incorporation being reduced by 35% when compared with the situation in the control medium containing 0.4 mM serine.

### [U-$^{14}$C]Serine incorporation into glycogen

When 0.4 mM [U-$^{14}$C]serine (5 $\mu$Ci/$\mu$mol) was used as a precursor of glycogen during short-term labelling experiments, no detectable glycogen labelling could be measured whatever the time of the culture. This flux measurement indicates that gluconeogenesis and glycogenesis from serine do not proceed in ZHC cells under the conditions considered, so that glucose-derived intermediates channelled to serine synthesis could not be ultimately diverted towards the pathway of glycogen synthesis.

## DISCUSSION

When replated at low density, fast-growing Zajdela hepatoma cultured (ZHC) cells exhibited an exponential phase of multiplication which was characterized by the mobilization of glycogen stores. This inverse relationship between cell density and glycogen content, which is in good agreement with previous observations [26], has also been reported for several human tumour cell lines [27,28]. The high cell glycogen concentration observed just after replating is consistent with the observations made in various tumoral cells characterized by high rates of glucose consumption [28,29]. These values of glycogen content were significantly higher than those previously reported in confluent ZHC cells (500 versus 80 $\mu$g/mg of protein) [12]. This probably reflects different features between the heterogeneous cell system originally described and the subclone used in the present study. Thus, the former required a concentration of glucose of 45 mM to achieve its growth [11] while the latter is able to proliferate in a 5.5 mM glucose-containing medium (Figure 1).

Pulse-labelling experiments were performed in order to investigate the quantitative contribution to cell metabolism of [$^{14}$C]glucose taken up from the medium when present at a physiological concentration. Glucose carbon incorporation into glycogen was inversely correlated with cell multiplication, since it remained constant during the exponential phase of growth and markedly increased during the slow-growing period. The rate of incorporation was then in the range of that determined in fetal [6,30,31] and adult [32–34] rat hepatocyte cultures. The high production of lactate over the 4 days of the culture reflected the important glycolytic activity that is characteristic of various hepatoma cells during rapid growth [35], and suggests that part of the glucose taken up was directed towards metabolic pathways other than glycogen formation. Indeed, glucose carbons were found to be substantially incorporated into proteins and nucleic acids. Comparison of label incorporation from [1-$^{14}$C]glucose and from [U-$^{14}$C]glucose allowed us to eliminate the possibility that a significant part (i.e. $> 10\%$) of the radioactivity in nucleotides was actually present in ribose and deoxyribose units formed through the oxidative limb of the pentose-phosphate pathway [21]. Thus a major contribution of ribose and deoxyribose to our measurements would only be possible if ribose 5-phosphate formation were to proceed essentially through the non-oxidative limb of the pentose-phosphate pathway, which seems unlikely. Inasmuch as C-1 from glucose may be incorporated into thymidylate and also into purine bases through conversion of labelled 5,10-methylenetetrahydrofolate into 10-formyltetrahydrofolate [22], the data do not allow us to distinguish between DNA- and RNA-base labelling. Nevertheless, the results indicate that glucose metabolism actually interfered with that of amino acids and supports the hypothesis of an additional role of glycolysis in furnishing nucleotide precursors [2]. The rate per cell of this glucose → amino acid → nucleic acids pathway predominated over that of glucose carbon incorporation into glycogen in the fast-growing phase, which is consistent with the increased proliferative capacity required in the exponential phase of cell multiplication. To our knowledge, these metabolic flux measurements provide the first demonstration that glucose-derived intermediates contribute to the assembly of nucleotide bases in liver-derived cells.

Concerning insulin action, the absence of an effect on glucose metabolism in the early phase of cell growth was not due to a time-dependent development of the cell sensitivity to the hormone, since glucose carbon incorporation into both lipids and glycogen is stimulated in confluent ZHC cells 1 day after replating [13]. The appearance of the glycogenic effect of the hormone after 72 h therefore probably depends on cell density. The absence of an insulin effect on glucose incorporation into nucleotides (this paper) as well as on glucose transport [13] suggests that the resurgence of the glycogenic response to insulin in ZHC cells could be linked to the growth-dependent differentiation process [12], leading to a switch of glucose utilization for glycogen formation at the expense of its diversion towards the provision of precursors required for proliferation.

Because of the branching of glycolysis to the 'phosphorylated pathway' of serine biosynthesis [1], serine and its direct metabolite glycine are the most probable intermediates of glucose incorporation into nucleic acid bases. Serine consumption peaked between 24 and 72 h, which is in agreement with the increased demand for nucleotide precursors during the exponential phase of cell multiplication. On the other hand, the presence of 0.4 mM glycine in the fresh medium suggests that the net glycine release measured after each 24-h period resulted from its transient accumulation inside the cell, which contrasts with the net glycine uptake commonly reported with non-proliferating rat hepatocytes [36–38]. The production of labelled glycine by cells incubated with [U-$^{14}$C]glucose indicates that the pathway from glycolysis to glycine is operative in this hepatoma cell line. These data are consistent with previous studies reporting a greater activity of the 'phosphorylated pathway' of serine biosynthesis in various hepatoma cells than in the corresponding normal tissue [9,39,40]. The high accumulation of labelled glycine observed during the first 24 h of culture suggests that the coupling of serine biosynthesis with its utilization as a precursor of thymidylate was operative in the early phase of cell multiplication [2]. This also indicates that the production of glycine by the cells exceeded its requirement as a precursor of nucleic acids, proteins, etc. Thus, the decreased rate of release of glucose-derived glycine (Table 1) as a function of cell growth was correlated with the increased rate of glucose carbon incorporation into nucleotide bases (Figure 2). This is consistent with the role of glycine as a precursor of both DNA and RNA purine bases.

The use of DL-cycloserine as an inhibitor of transaminases was particularly suitable since this agent does not enter the mitochondria [24] and does not alter cell growth, at least during short-term experiments (Figure 3). Besides, in a previous study, the action of cycloserine in cultured fetal hepatocytes allowed us to reveal the contribution of the serine-biosynthesis-mediated alternative pathway of glycogen synthesis from glucose [7]. The results presented herein show that cycloserine actually blocks serine and glycine biosynthesis from glycolysis (Figure 3), and are consistent with an inhibition of the 'phosphorylated pathway' of serine formation at the level of the cytoplasm-located phosphoserine aminotransferase [2]. The inhibitory effect of DL-cycloserine on nucleotide labelling from [U-$^{14}$C]glucose (Figure 4) further supports the role of serine and glycine as intermediates of glucose carbon incorporation into nucleic acids. As mentioned above, the release of labelled serine and glycine into the medium reflects their accumulation inside the cell, which results from the equilibrium between the synthesis of these substrates from glycolysis and their utilization by the cells as precursors of end metabolic products, including incorporation into nucleotide bases. This would explain why nucleotide labelling was not completely prevented under conditions where serine and glycine release was. The inability of cycloserine to totally inhibit glucose carbon incorporation into nucleotides is difficult to explain in the absence of informative data concerning the kinetic parameters exhibited by phosphoserine aminotransferase regarding this agent. Furthermore, the possible reaction of cycloserine with other transaminases would most likely complicate the interpretation. While the overestimation of nucleic acid base labelling by ribose and deoxyribose units is unlikely (see above), such an hypothesis cannot be totally excluded. However that may be, the results show that at least 70% (and probably more) of label incorporation into the nucleotide base fraction (Figure 4) resulted from the intermediary conversion of glucose into amine compounds.

The biosynthesis of serine from glucose prior to incorporation into nucleic acids might be expected to be affected by extracellular serine concentration due to feedback inhibition exerted by serine on the irreversible step catalysed by the phosphoserine phosphatase [25], the activity of which is increased in many tumours [2]. The maximal inhibitory effect on nucleotide labelling from [U-$^{14}$C]glucose was observed with 5 mM serine (Figure 4), which is consistent with the parameters of serine phosphatase inhibition by L-serine reported from *in vitro* measurements [39,41,42]. From these studies, the $K_i$ value for this non-competitive inhibition corresponds to the concentration of serine in the fresh culture medium used in the present study ($\approx 0.4$ mM), which would explain the relatively moderate (35%) effect observed. In ad-

dition, the transfer of phosphoryl group from phosphoserine to serine would explain the observation that serine labelling still occurred in the presence of high serine concentrations [43], suggesting that the effect of the amino acid mainly resulted from isotopic dilution. Nevertheless, although the effect of serine was most likely underestimated, the role of other amines as intermediates of glucose carbon incorporation into nucleotide bases may be evoked. Aspartate, which participates in the assembly of pyrimidines, appears as a potential candidate due to the possible labelling of its precursor oxaloacetate through the citric acid cycle. At all events, the results reinforce the hypothesis that a glucose → serine + glycine → nucleotides pathway contributes to the increased proliferative capacities of the cell.

Another question that arises from these data is the analogy between hepatoma cells and fetal hepatocytes, in which serine can be biosynthesized *de novo* from glucose [7]. While the hypothesis that both types of cells could exhibit similar interrelationships in glucose and serine metabolism is quite attractive, at least two major differences must be pointed out. First, the present results show a net glycine production correlated to a net serine uptake, whereas an inverse pattern has been reported with 18-day fetal-rat hepatocytes [44]. Assuming that the glycine accumulation resulted from the diversion of serine towards the pathways of DNA replication, this discrepancy can be ascribed to the faster multiplication of proliferating hepatoma cells than of fetal hepatocytes. Secondly, serine can be channelled to the gluconeogenic pathway and consequently to glycogenesis via the serine aminotransferase pathway in late-fetal hepatocytes [5–7], while the gluconeogenic pathways from serine appear to be inoperative in terms of enzyme activities [2,9] as well as of metabolic fluxes (this paper) in hepatomas. The direct consequence of this one-way flux from glycolysis to serine in proliferating hepatoma cells is an increased provision of protein and nucleotide precursors at the expense of glycogen storage. By contrast, the development of late-fetal hepatocytes is coincident with active glycogen storage, so that a part of serine formed *de novo* from glycolytic intermediates is re-converted back into glucose 6-phosphate and then channelled towards glycogenesis, as revealed by the alternative pathway of glycogen synthesis from glucose [7].

Further studies will be required to define the specific mechanisms (and regulators) controlling the interrelationships in the pathways of glucose and serine metabolism in fetal and tumoral cells. The possibility that the conversion of glycolytic substrates into amine compounds may account for the action of glucose on the programme of some tumour cell differentiation [28] remains to be specified. Nevertheless, the present study reinforces the hypothesis that a coupling of serine and glycine biosynthesis from glycolysis with their diversion towards nucleotide formation is involved in the growth advantage of cancer cells when compared with those constituting normal tissues.

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