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# Role of serine biosynthesis and its utilization in the alternative pathway from glucose to glycogen during the response to insulin in cultured foetal-rat hepatocytes

Hervé BISMUT* and Christiane PLAS

Laboratoire de Biologie, U.E.R. d'Odontologie, Université Paris 7, Institut des Cordeliers, 15 rue de l'Ecole-de-Médecine, 75006 Paris, France

The role of serine as a possible intermediate of the alternative pathway from glucose to glycogen was investigated under basal and insulin-stimulated conditions in 18-day cultured foetal-rat hepatocytes because these cells cannot use pyruvate-derived metabolites [Bismut & Plas (1989) Biochem. J. **263**, 889–895]. Incubation of cells with [U-$^{14}$C]glucose for 24 h led to a release of labelled serine in the medium concomitantly with a net serine production (100 nmol/24 h per culture). The rate of [$^{14}$C]serine formation (close to 3 nmol/h per culture) indicated that a large part of newly formed serine originated from glucose. When short-term experiments were performed at day 2, glycogen labelling from [U-$^{14}$C]serine or [U-$^{14}$C]glycine, which was increased 3-fold by insulin after 2 h, evidenced their participation as glycogenic precursors. When a double-isotope procedure with [U-$^{14}$C,3-$^{3}$H]glucose was used, the direct and the alternative pathways from glucose were found to contribute to glycogenesis by 75 and 25% respectively. Cycloserine (18 mM), a transaminase inhibitor, strongly inhibited glycogen labelling from [U-$^{14}$C]serine while producing a 70% increase in glucose incorporation by the alternative pathway, in both the presence and the absence of insulin. The inhibitor had no effect on the direct pathway from glucose to glycogen. Supplementation with 1 mM-hydroxypyruvate, a serine-derived metabolite, did not affect direct glucose incorporation, whereas the alternative pathway was stimulated whether insulin was present or not. These results indicate that the sequence glucose → serine → glycogen is operative in cultured foetal hepatocytes. The alternative pathway interferes with hydroxypyruvate utilization, and is likely mediated by the serine aminotransferase pathway, independently of the acute glycogenic action of insulin.

## INTRODUCTION

The role of glucose as a precursor of glycogen formation has been a subject of controversy for many years. Findings over the last decade indicate that in the postprandial phase glucose is not directly incorporated into hepatic glycogen but is first degraded to $C_3$ compounds, presumably in lactate, the site of lactate formation (hepatic or extrahepatic) remaining to be established (for reviews, see Katz & McGarry, 1984; Pilkis *et al.*, 1985; McGarry *et al.*, 1987). Studies on cultured adult (Spence & Koudelka, 1985; Parniak & Kalant, 1985; Salhanick *et al.*, 1989) and foetal (Bismut & Plas, 1989) rat hepatocytes have shown that the classical direct pathway of glucose incorporation into glycogen functions concomitantly with an indirect one mediated by triose phosphate formation, and that both direct and indirect pathways are stimulated by insulin. As regards foetal hepatocytes, the indirect pathway, which involves one-third of the glucose used for glycogen synthesis, is not mediated by the formation of pyruvate-derived metabolites (Bismut & Plas, 1989). Indeed, the poor activity of phosphoenolpyruvate carboxykinase (PEPCK) prevents foetal hepatocytes from using gluconeogenic intermediates which enter at the level of lactate/pyruvate (Hanson *et al.*, 1975; Girard, 1986; Bismut & Plas, 1989). Thus it has been concluded that the indirect pathway, termed 'alternative', implies substrates bypassing the step catalysed by PEPCK, and the implication of serine as a possible intermediate of glucose incorporation into glycogen has been postulated (Bismut & Plas, 1989). In adult hepatocytes, serine, and also its direct precursor glycine, can enter gluconeogenesis at the level both of pyruvate via the serine dehydratase pathway and of 2-phosphoglycerate via the serine aminotransferase pathway (for review, see Snell, 1984), the latter being the only possible route for glycogen synthesis from serine and glycine in foetal hepatocytes.

The aim of the present paper was to correlate the pathways involved in serine biosynthesis and its utilization with the alternative pathway from glucose to glycogen in 18-day cultured foetal-rat hepatocytes. Doubly labelled glucose was used to estimate the contributions of the direct and the alternative pathways from glucose to glycogen in the presence or absence of transaminase inhibitors. The results indicate that the pathways of serine biosynthesis from glucose and of serine incorporation into glycogen were operative. The alternative pathway from glucose to glycogen was specifically stimulated by transaminase inhibitors and interfered with the utilization of hydroxypyruvate, an intermediate of the aminotransferase pathway of serine utilization.

## MATERIALS AND METHODS

### Materials

Insulin was purchased from Novo Laboratories. L-Serine, L-glycine, DL-cycloserine and β-hydroxypyruvate were obtained from Sigma. D-[U-$^{14}$C]Glucose was from New England Nuclear, and L-[U-$^{14}$C]serine, [U-$^{14}$C]glycine and D-[3-$^{3}$H]glucose were from Amersham. Radiochemical purity of D-[3-$^{3}$H]glucose was checked by t.l.c. in formic acid/butan-1-one/butan-1-ol/water (3:6:8:3, by vol.) and in propan-2-ol/water (7:3, v/v), and glucose was found to correspond to 95 and 99% of the labelled compounds respectively. The sources of other materials have been specified previously (Plas & Nunez, 1976).

Abbreviation used: PEPCK, phosphoenolpyruvate carboxykinase.

* To whom correspondence should be addressed.

### Culture procedure

Primary cultures of hepatocytes were obtained from 18-day rat foetuses (Sprague–Dawley) as described previously (Plas *et al.*, 1973). After mild trypsin treatment, the isolated cells were plated on a collagen substratum to which only the hepatocytes adhered, and after 6 h the non-adhering haematopoietic cells were removed. At this point, the culture medium (1 ml/well) was replaced and renewed after 24 h. This culture medium consisted of NCTC 109 medium (Evans *et al.*, 1964) containing a glucose concentration of about 5.5 mM and supplemented with 10 $\mu$M-cortisol and 10% (w/v) foetal-calf serum. Unless otherwise stated, experiments were performed after 2 days of culture in the presence of cortisol, by which time the glycogenic effect of insulin is fully expressed (Plas & Nunez, 1976). The glucose concentration in the medium was close to 4 mM, which corresponds to the glycaemia of the rat foetus at the end of gestation.

### Glycogen and metabolite studies

Glycogen content and glycogen labelling were measured as previously described (Plas *et al.*, 1973, 1979). In 24 h-labelling experiments, [U-$^{14}$C]glucose (2 $\mu$Ci/mg) together with 10 nM-insulin or its solvent (HCl, 2.5 $\mu$M final concn. in the medium) were added at day 1 when the medium was replaced by a fresh one. After various times of incubation, the medium was collected, and glucose and serine concentrations, as well as their specific radioactivities, were determined as described below. Serine production from glucose was calculated as [radioactivity in the medium incorporated into serine ($\mu$Ci/ml)/sp. radioactivity of medium glucose at the start of the labelling ($\mu$Ci/nmol)] $\times$ 2, and was expressed in nmol of serine per culture (1 ml).

Short-labelling experiments were performed after 2 days of culture. The labelled substrates and the agents to be tested were added together with 10 nM-insulin or its solvent, and the radioactivity present in glycogen was determined after 2 h of incubation. When glucose was used as a labelled substrate, the cells of the same dish were exposed to both [U-$^{14}$C]glucose (1 $\mu$Ci/mg) and [3-$^{3}$H]glucose (2 $\mu$Ci/mg). Incorporations of $^{3}$H and $^{14}$C into glycogen were measured simultaneously in a Beckman LS 7500 liquid-scintillation counter (dual-label d.p.m.) with appropriate channel discriminators. The use of doubly labelled glucose allowed assessment of the contributions of the direct and the alternative pathways from glucose to glycogen, as detritiation of [3-$^{3}$H]glucose during glycolysis occurs at the level of triose phosphate formation (Katz & Rognstad, 1976), the flux through the pentose phosphate pathway being negligible in this cell system (Bismut & Plas, 1989). $^{14}$C incorporation reflected both the direct and the alternative pathways from glucose to glycogen, whereas $^{3}$H incorporation only corresponded to glucose incorporated by the direct pathway. Consequently, the rate of glucose incorporation by the alternative pathway was the difference between $^{14}$C and $^{3}$H incorporations into glycogen.

### Determination of metabolites

[$^{14}$C]Serine and [U-$^{14}$C]glucose in the 24 h-conditioned medium were separated from other labelled compounds by t.l.c. Samples of medium were applied on silica gel G plates (Merck) after deproteinization with $HClO_4$ (0.33 M), and migration was performed in ethanol/water (7:3, v/v). The plates were then dried and subjected to autoradiography (Kodak X-OMAT AR) by using an Enhancer spray (New England Nuclear). The areas co-migrating with authentic standards were scraped off and the radioactivity was determined by scintillation counting. Glucose concentration in the medium was determined by the glucose oxidase procedure (Boehringer-Mannheim) after deproteinization with $Ba(OH)_2/ZnSO_4$. Serine and glycine concentrations in the medium were measured by using ion-exchange chromatography as previously described (Lemonnier *et al.*, 1976).

### Definitions

Each protocol involved at least three independent experiments performed on different cell preparations. Data are presented as means $\pm$ S.E.M. for the numbers of independent experiments indicated. For statistical analyses, Student's *t* test for paired samples was used, with treated cultures and the corresponding controls for *n* independent experiments. When representative experiments are presented, each symbol in the Figures corresponds to different triplicate cultures, and the range of errors is indicated by S.D. values of the triplicate measurements from the shown experiment. Glycogen labelling was expressed as nmol of glucosyl units in glycogen/mg of cell protein. The cell population of a culture well was of the order of $0.8 \times 10^6$ hepatocytes, which corresponds to 310 $\mu$g of protein and to 2.10 mg of wet liver. In order to express the glycogenic response to insulin, a 'stimulation index' was used, defined as the ratio nmol of glucosyl units in glycogen per mg of cell protein in treated cultures divided by nmol of glucosyl units in glycogen per mg of cell protein in control cultures.

## RESULTS

### Production of serine from glucose and utilization of serine for glycogen synthesis in the absence and presence of insulin

In order to determine to what extent the pathways of serine production from glucose were operative, hepatocytes were in-

**Table 1. Production of labelled serine in the medium from [U-$^{14}$C]glucose in the absence and presence of insulin**

After 24 h of culture, the medium was removed and replaced by fresh medium. At the same time, [U-$^{14}$C]glucose (2 $\mu$Ci/mg) was added together with 10 nM-insulin or its solvent, and the medium was collected 24 h later. Glucose and serine concentrations in fresh and 24 h-conditioned media and their specific radioactivities were determined as described in the Materials and methods section. Results are presented as means $\pm$ S.E.M. for four experiments performed with different cell preparations: $*P < 0.001$ compared with corresponding control in the absence of insulin.

| Time of labelling | Insulin | Glycogen content (nmol of glucose/mg of protein) | Concn. in the medium (nmol/ml) | | Sp. radioactivity in the medium (nCi/nmol) | |
|---|---|---|---|---|---|---|
| | | | Glucose | Serine | Glucose | Serine |
| 0 | | 440 $\pm$ 60 | 5900 $\pm$ 260 | 100 $\pm$ 5 | 0.343 $\pm$ 0.014 | – |
| 24 h | – | 840 $\pm$ 80 | 4290 $\pm$ 110 | 223 $\pm$ 16 | 0.355 $\pm$ 0.011 | 0.077 $\pm$ 0.008 |
| 24 h | + | 1430 $\pm$ 60* | 4180 $\pm$ 130 | 229 $\pm$ 10 | 0.368 $\pm$ 0.012 | 0.072 $\pm$ 0.008 |

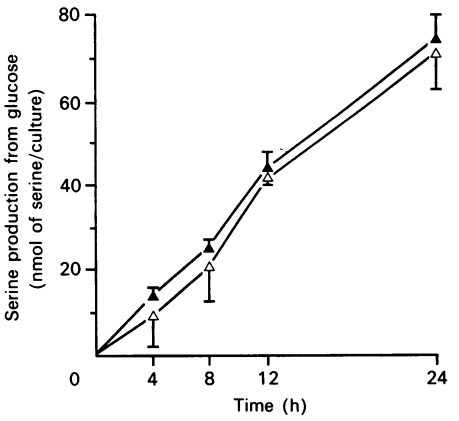


**Fig. 1. Time course of serine production from glucose in the presence and absence of insulin**

After 24 h of culture, the medium was removed and replaced by fresh medium. At the same time, [U-$^{14}$C]glucose (2 $\mu$Ci/mg) was added together with 10 nM-insulin or its solvent, and the medium was collected after the times indicated. Labelled serine production was determined as described in the Materials and methods section. Serine production from glucose in the presence (▲) or absence (△) of insulin is shown. Results are presented as means ± S.D. for triplicate measurements from the representative experiment shown.

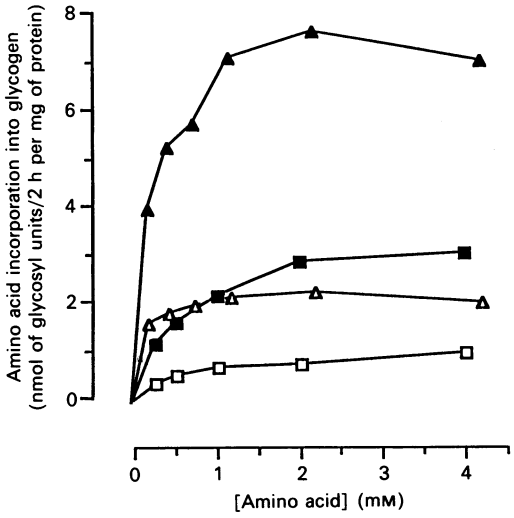


**Fig. 2. Glycogen synthesis from [U-$^{14}$C]serine and [U-$^{14}$C]glycine in the presence and absence of insulin**

After 2 days of culture, the medium containing 0.20 mM-serine and 0.04 mM-glycine was supplemented with [U-$^{14}$C]serine (24 $\mu$Ci/mg) or [U-$^{14}$C]glycine (13 $\mu$Ci/mg) at the concentrations indicated, together with 10 nM-insulin or its solvent. The radioactivity incorporated into glycogen was determined 2 h later. Cultures supplemented with [U-$^{14}$C]serine (△, ▲) or [U-$^{14}$C]glycine (□, ■) together with insulin (▲, ■) or its solvent (△, □) are shown. 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.

cubated for 24 h in the presence of [U-$^{14}$C]glucose, 10 nM-insulin being present or absent. The concomitant presence of the hormone gave a 2.5-fold increase in glycogen synthesized during this period (Table 1). Measurements of glucose and serine concentrations in the medium after 24 h revealed a net production of serine by the cells [129 ± 10 and 123 ± 15 nmol/24 h per culture ($n$ = 4) in the presence and absence of insulin respectively] concomitantly with a net glucose consumption [1720 ± 310 and 1610 ± 320 nmol/24 h per culture ($n$ = 4) in the presence and absence of insulin respectively]. Some of the serine in the conditioned medium appeared to be labelled, with a specific radioactivity close to 0.08 $\mu$Ci/$\mu$mol, in both the presence and the absence of insulin. Time-course experiments showed that the glucose specific radioactivity remained constant, indicating that the reverse flux of glucose from the cell back to the medium was not operative; by contrast, serine specific radioactivity increased progressively and tended to reach a plateau between 12 and 24 h (results not shown). The specific radioactivity of serine determined after 24 h gave a minimum estimate of the proportion of newly synthesized serine originating from glucose. Indeed, the presence of extracellular serine at the start of incubation diluted the specific radioactivity of serine produced by the cells, so that it was difficult to determine the rate of serine production from glucose, especially in the early period of labelling. Yet it was possible to see a continuous increase during the 24 h of incubation (Fig. 1), which corresponded to an apparent rate of synthesis close to 3 nmol of serine/h per culture, in both the presence and the absence of insulin. These results revealed that a large part of the serine released by the cells originated from glucose in the medium and that insulin had no effect on the intracellular flux from glucose to serine.

At day 2 of culture, incorporations of $^{14}$C into glycogen from [U-$^{14}$C]serine, and also from [U-$^{14}$C]glycine as a direct precursor of serine, were determined after 2 h in the presence and absence of insulin. Glycogen labelling from [U-$^{14}$C]serine was clearly dependent on the dose of serine used, $^{14}$C incorporation reaching a maximal value (close to 2 nmol of glucosyl units/2 h per mg of protein) at 1 mM (Fig. 2). The same pattern was observed in the presence of insulin. The hormone exerted a clear stimulatory effect on label incorporation at all the concentrations of serine tested, the 'stimulation index' being 2.9 ± 0.3 ($n$ = 7) at 1 mM-serine. The high rate of serine production at day 2 (Fig. 1) was responsible for a dilution of the labelled serine added to the medium, so that $^{14}$C incorporation into glycogen only represented the minimum participation of serine in glycogen synthesis. When tested as a glycogenic precursor, [U-$^{14}$C]glycine was found to be effective for the same range of concentration, although maximal glycogen labelling represented about 40% of that measured by using labelled serine under both basal and insulin-stimulated conditions. The effect of the hormone was similar to that obtained with labelled serine, with a 'stimulation index' close to 3 at 1 mM-glycine. These results reflect the capacity of the cells to use glycine- and serine-derived metabolites as glycogenic precursors.

### Effects of cycloserine on glycogen synthesis from [U-$^{14}$C]serine and [U-$^{14}$C,3-$^{3}$H]glucose in the presence and absence of insulin

The action of cycloserine, a transaminase inhibitor (Brosnan *et al.*, 1970; Edmondson *et al.*, 1977), was tested on glycogen synthesis, since a pathway from glucose to glycogen mediated by serine formation would require a transamination step. The addition of increasing concentrations of cycloserine progressively decreased glycogen labelling from [U-$^{14}$C]serine determined 2 h later (Fig. 3*a*). Incorporation from [U-$^{14}$C]serine was decreased by 50% with 3 mM-cycloserine in both the absence and presence of insulin, a total inhibition being obtained at 18 mM-cycloserine under both conditions. The drastic effect of the inhibitor at these concentrations on [U-$^{14}$C]serine incorporation was not accompanied with a significant modification of glycogen content, except that under insulin-stimulated conditions glycogen content was further increased in the presence of 18 mM-cycloserine.

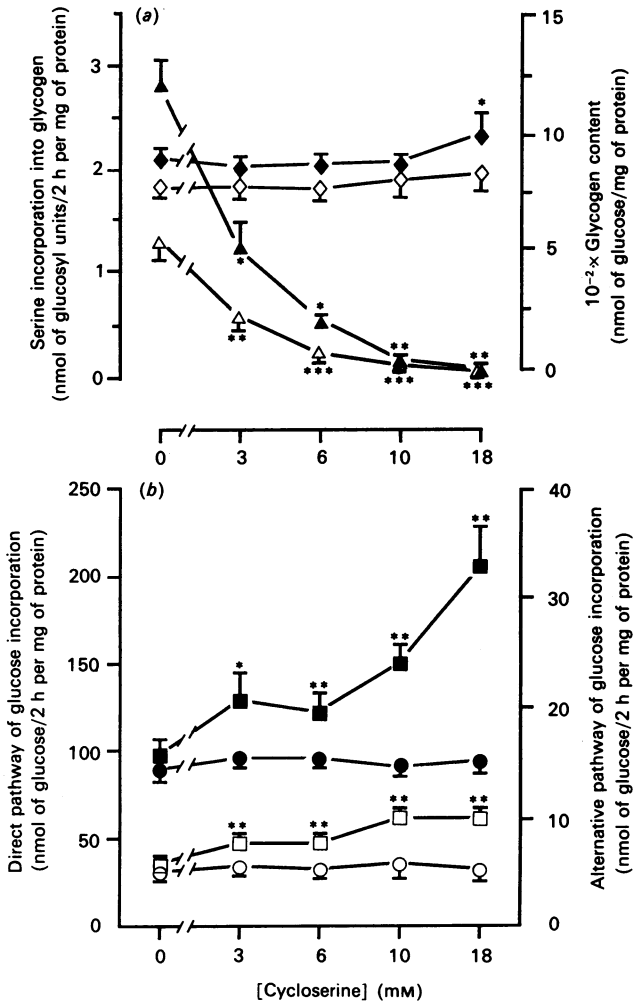


**Fig. 3. Effect of cycloserine on glycogen labelling from [U-$^{14}$C]serine and [3-$^{3}$H,U-$^{14}$C]glucose in the presence and absence of insulin**

After 2 days of culture, the medium containing 0.2 mM-serine and 4 mM-glucose was supplemented with [U-$^{14}$C]serine (24 $\mu$Ci/mg) (*a*) or with [U-$^{14}$C]glucose (1 $\mu$Ci/mg) and [3-$^{3}$H]glucose (2 $\mu$Ci/mg) (*b*) together with 10 nM-insulin or its solvent. At the same time, cycloserine was added at the concentrations indicated. Glycogen content (*a*) and radioactivity incorporated into glycogen from labelled serine (*a*) or from doubly labelled glucose (*b*) were determined 2 h later. Glucose incorporations by the direct and the alternative pathways were measured as described in the Materials and methods section. Glycogen content (◇, ◆) and incorporation from [U-$^{14}$C]serine (△, ▲) or [U-$^{14}$C,3-$^{3}$H]glucose by the direct (○, ●) and the alternative (□, ■) pathways in cultures supplemented with insulin (◆, ▲, ●, ■) or its solvent (◇, △, ○, □) are shown. Results are presented as means ± S.E.M. for three experiments performed with different cell preparations: $*P < 0.02$, $**P < 0.01$ and $***P < 0.001$ compared with corresponding control in the absence of cycloserine.

[U-$^{14}$C,3-$^{3}$H]Glucose was used to estimate the relative contributions of the direct and the alternative pathways from glucose to glycogen, as detritiation of [3-$^{3}$H]glucose occurs during glycolysis at the level of triose phosphate formation (Katz & Rognstad, 1976; Newgard *et al.*, 1983). Since the specific radioactivity of labelled glucose in the medium remains constant throughout the culture period (Table 1), it can be considered that glucose incorporation during short-term labelling experiments corresponded to a net carbon transfer into glycogen. In the present study, the ($^{3}$H/$^{14}$C in glycogen)/($^{3}$H/$^{14}$C in glucose) ratio determined in basal conditions was 0.77 ± 0.03 ($n = 7$), indicating that the contribution of the alternative pathway was about 25% of glucose incorporation. In the absence of any agent, glucose incorporation by the alternative pathway was close to 6 nmol of glucose/2 h per mg of protein (Fig. 3*b*), which corresponded to about 2 nmol of serine equivalents/2 h per culture. When cycloserine was added together with [U-$^{14}$C,3-$^{3}$H]glucose under basal conditions, the direct pathway from glucose to glycogen was not modified. By contrast, a stimulation of the alternative pathway was revealed, which corresponded to a 70% increase with 10 mM- and 18 mM-cycloserine. In the presence of insulin a 'stimulation index' close to 3.5 was found for both direct and alternative pathways. Similar results to those found in the absence of the hormone were obtained after supplementation with cycloserine, yet with a more pronounced stimulation of the alternative pathway.

### Effect of hydroxypyruvate on glycogen synthesis from [3-$^{3}$H,U-$^{14}$C]glucose in the absence and presence of insulin

In order to characterize the intermediary metabolites involved in the alternative pathway from glucose to glycogen, the role of hydroxypyruvate was studied as an intermediary metabolite of the serine aminotransferase pathway of serine utilization (Williamson & Ellington, 1975). As shown in Table 2, the presence of hydroxypyruvate did not modify the direct incorporation of glucose into glycogen, whether insulin was present or not. By contrast, a significant stimulation of the alternative pathway was obtained with 1 mM-hydroxypyruvate, a concentration at which glycogen content was not affected after 2 h. This specific stimulatory effect corresponded to increases of 45 and 25% in the absence and presence of insulin respectively. When hydroxypyruvate was tested at 2 mM, the rate of glucose incorporation by each pathway remained unchanged when compared with the corresponding value in the absence of hydroxypyruvate, despite a significant increase in glycogen content in the simultaneous presence of insulin.

## DISCUSSION

Serine-derived metabolites were postulated as possible intermediates of the alternative pathway from glucose to glycogen in cultured foetal hepatocytes, a cell system in which PEPCK is poorly active (Bismut & Plas, 1989). A net release of serine into the medium by cultured foetal hepatocytes was obtained in the presence as well as in the absence of insulin, whose major part originated from glucose. This direct flux measurement clearly shows the capacity for serine biosynthesis *de novo* from glycolytic intermediates in foetal hepatocytes. It is the first report that serine synthesis actually occurs in foetal rat liver, in agreement with the relevant enzyme activities measured *in vitro* (Johnson *et al.*, 1964; Jamdar & Greengard, 1969; Knox *et al.*, 1969; Snell, 1980). On the other hand, the efficient and insulin-stimulated incorporations of serine into glycogen, largely underestimated by the net serine production over the culture period, expressed the capacity for foetal hepatocytes to divert serine-derived metabolites towards the pathways of glycogen formation. This process is likely mediated by the aminotransferase pathway, since the dehydratase pathway of serine utilization would require a PEPCK activity (for review, see Snell, 1984). In foetal hepatocytes, serine utilization for glycogen formation has been shown not to be inhibited when PEPCK was blocked by 3-mercaptopicolinic acid (Bismut & Plas, 1989), in accordance with the fact that foetal liver is devoid of serine dehydratase activity, whereas serine aminotransferase activity represents 30% of the adult value (Snell, 1980). The net production of serine by foetal hepatocytes reveals that their high capacity to form serine *de novo* could be used to supply it to extrahepatic tissues, in which

**Table 2. Effect of hydroxypyruvate on glycogen synthesis from [3-$^3$H,U-$^{14}$C]glucose in the absence and presence of insulin**

After 2 days of culture, the medium containing 4 mM-glucose was supplemented with [U-$^{14}$C]glucose (1 $\mu$Ci/mg) and [3-$^3$H]glucose (2 $\mu$Ci/mg) together with 10 nM-insulin or its solvent. At the same time, hydroxypyruvate was added at the concentrations indicated. Glycogen content and radioactivity incorporated into glycogen were determined 2 h later. Glucose incorporations by the direct and the alternative pathways were estimated as described in the Materials and methods section. Results are presented as means ± S.E.M. for five experiments performed with different cell preparations: $*P < 0.02$ and $**P < 0.01$ compared with corresponding control in the absence of hydroxypyruvate.

| [Hydroxypyruvate] (mM) | Insulin | Glycogen content (nmol of glucose/ mg of protein) | Glucose incorporation into glycogen (nmol of glucose/2 h per mg of protein) | |
|---|---|---|---|---|
| | | | Direct pathway | Alternative pathway |
| 0 | − | 590±80 | 20.8±3.0 | 4.4±0.2 |
| | + | 760±80 | 69.1±8.0 | 12.8±0.8 |
| 1 | − | 620±80 | 20.9±3.0 | 6.4±0.6** |
| | + | 815±100 | 69.6±10.2 | 16.1±1.6* |
| 2 | − | 650±90 | 21.2±3.8 | 4.9±0.7 |
| | + | 920±100* | 63.6±11.3 | 11.9±3.4 |

serine biosynthesis is poorly active in late-foetal life (Jamdar & Greengard, 1969; Snell, 1984). All these results indicate that the sequence glucose → serine → glycogen is operative in cultured foetal hepatocytes. On the other hand, glycine incorporation into glycogen permitted the assumption that in foetal hepatocytes glycine is protected from oxidative pathways and is preferentially utilized for anabolic processes such as glycogen synthesis. Though glycine appears to be glucogenic in the adult rat *in vivo* (Hetenyi *et al.*, 1988), glucose formation from the amino acid is negligible in hepatocytes isolated from fed adult rats (Beliveau & Freedland, 1982; Rémésy *et al.*, 1983).

The use of doubly labelled glucose allowed definition of the trioses-mediated alternative pathway involved in glycogen synthesis from glucose in cultured foetal hepatocytes (Bismut & Plas, 1989). In the present study, the alternative pathway represented one-quarter of the total glucose incorporation into glycogen. The rate of $^{14}$C incorporation from glucose into serine suggests that this process largely exceeded glucose incorporation into glycogen by the alternative pathway. This implies that serine biosynthesis from glucose operated at a requisite rate to support the hypothesis of a pathway from glucose to glycogen mediated by serine formation.

The $C_3$-compounds-mediated alternative pathway from glucose to glycogen was clearly stimulated by cycloserine, but the direct pathway was not altered. The inhibitor is expected only to block cytoplasmic steps, since it cannot reach intramitochondrial transaminases (Meijer & Van Dam, 1974). Thus it is likely that cycloserine did not affect serine aminotransferase, which is a mitochondrial transaminase, whereas it may inhibit the phosphorylated pathway of serine biosynthesis at the level of the cytoplasm-located phosphoserine aminotransferase (Snell, 1975), leading to the accumulation of phosphohydroxypyruvate at the expense of serine formation. Because of the equilibrium positions of the enzymes of serine biosynthesis in foetal liver (Fell & Snell, 1988), the flux in the serine-synthesis pathway would reverse towards glycolysis and glycogenesis. Thus the increased availability of glucose-derived $C_3$ compounds for glycogenesis may account for the stimulation of the alternative pathway of glycogen formation from glucose in foetal hepatocytes. Furthermore, the reverse reaction from phosphohydroxypyruvate to 3-phosphoglycerate would prevent the label from being diluted by going through the serine pool on its way to glycogen. One can postulate that in cultured foetal hepatocytes glucose-derived $C_3$ compounds are diverted to metabolic pathways mediated by serine formation, but that only one proportion of newly formed serine regenerates glucose 6-phosphate to be ultimately incorporated into glycogen.

Assuming that cycloserine is unable to affect the serine aminotransferase activity, the decrease in serine utilization for glycogen formation in the presence of the inhibitor observed in cultured foetal hepatocytes would result from the stimulation of the alternative pathway from glucose, under which conditions serine might be channelled to metabolic pathways other than glycogenesis. Besides, the dilution of the label by glucose-derived $C_3$ compounds diverted to gluconeogenesis could also account for the decrease in [U-$^{14}$C]serine incorporation into glycogen. In adult hepatocytes, gluconeogenesis from serine, but not from D-glycerate, a serine-derived metabolite, has been found to be inhibited by cycloserine (Chen & Lardy, 1988). In these cells, the cytoplasm-located serine dehydratase contributes widely to serine utilization for gluconeogenesis (for review, see Snell, 1984), contrary to the situation in foetal hepatocytes.

Among the metabolic pathways through which transamination of glucose-derived metabolites can be expected, protein synthesis appears particularly interesting because of the increased provision of protein precursors required in foetal life. The substantial incorporation of glucose into proteins observed in cultured foetal hepatocytes (H. Bismut & C. Plas, unpublished work) is in favour of a role of the alternative pathway in diverting glucose and glycolytic substrates to metabolic pathways involved in protein synthesis. The role of serine as an intermediate would be in agreement with the inverse relationship between the protein content and the activity of the enzymes of the phosphorylated pathway of serine biosynthesis in the liver (Fallon, 1967; Hayashi *et al.*, 1975). Besides, serine utilization for nucleotide precursor formation, whose capacity is increased in late-foetal liver (Snell, 1980), could be metabolically coupled to serine biosynthesis *de novo* from glycolytic precursors, as has been shown in hepatoma cells (Snell *et al.*, 1987).

The intermediary formation of serine from glycolytic intermediates before incorporation into glycogen presents attractive features. When hydroxypyruvate was tested as an intermediate of the aminotransferase pathway of serine utilization at a concentration of 1 mM, a specific stimulatory action on the alternative pathway was obtained, whereas at 2 mM the effect was no longer observed. This indicates that glycogen synthesis from glucose-derived $C_3$ compounds interfered with the pathway involved in the utilization of hydroxypyruvate. A biphasic dose-dependent effect of hydroxypyruvate has been reported on

glucose formation in adult rat hepatocytes, in which the main metabolic fate of this compound is to enter gluconeogenesis (Sandoval & Sols, 1974; Williamson & Ellington, 1975). At 1 mM, hydroxypyruvate was thought to stimulate glucose formation through activation of glycerate dehydrogenase and increased gluconeogenesis, whereas at higher concentrations hydroxypyruvate appeared to be metabolized into lactate (Williamson & Ellington, 1975). In foetal hepatocytes, hydroxypyruvate from exogenous sources and from labelled glucose would be preferentially diverted to gluconeogenesis when tested at 1 mM, leading to the stimulation of the alternative pathway from glucose to glycogen. The inability of 2 mM-hydroxypyruvate to modify glycogen labelling would be due to the fact that lactate cannot be converted into glycogen, because of the low activity of PEPCK in foetal hepatocytes (Menuelle *et al.*, 1988; Bismut & Plas, 1989). The absence of effect of hydroxypyruvate on the direct pathway from glucose to glycogen rules out possible additional effects of this compound on glycogenesis, and is in favour of the alternative route mediated by the aminotransferase pathway of serine utilization. In conclusion, the present study suggests that the role of the alternative pathway from glucose to glycogen was to divert a proportion of $C_3$ glycolytic intermediates to the formation of amine compounds, whereas the rest would enter gluconeogenesis to be ultimately used for glycogen formation, most likely via the aminotransferase pathway of serine utilization.

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