Glycogen synthesis in amphibian oocytes: evidence for an indirect pathway

Eduardo KESSI*, Victoria GUIXÉ†, Ana PRELLER† and Tito URETA†‡

* Departamento de Ciencias Biológicas Animales, Facultad de Ciencias Veterinarias y Pecuarias, and † Departamento de Biología, Facultad de Ciencias, Universidad de Chile, Santiago, Chile

Glycogen is the main end product of glucose metabolism in amphibian oocytes. However, in the first few minutes after [$U-^{14}C$]glucose microinjection most of the label is found in lactate. The burst of lactate production and the shape of the time curves for the labelling of glucose 6-phosphate, fructose 6-phosphate, glucose 1-phosphate and glycogen suggest a precursor–product relationship of lactate with respect to glycogen and its intermediates. Expansion (by microinjection) of the pool of glycolytic intermediates, such as dihydroxyacetone phosphate, glyceraldehyde 3-phosphate, 3-phosphoglycerate or phosphoenolpyruvate, results in a marked decrease in [$U-^{14}C$]glucose incorporation into glycogen. After co-injection of doubly labelled glucoses, extensive detritiation (93 %) of the glucosyl units of glycogen was observed with [$2-^3H, U-^{14}C$]glucose and partial detritiation with [$3-^3H, U-$

^{14}C]glucose (34 %) or [$5-^3H, U-^{14}C$]glucose (46 %). After injection of [$6-^3H, U-^{14}C$]glucose, a small but significant and reproducible detritiation (13 %) in glycogen was observed. Co-injection of [$U-^{14}C$]glucose and 3-mercaptopicolinate resulted in marked inhibition of glycogen labelling. Half-maximal inhibition was observed at 0.58 mM 3-mercaptopicolinate, which agrees with the IC_{50} value (0.47 mM) for the inhibition *in vitro* of phosphoenolpyruvate carboxykinase activity. We conclude that in frog oocytes most of the glucosyl units are incorporated into glycogen by an indirect pathway involving breakdown of glucose to lactate, which is then converted into glycogen via gluconeogenesis. Both processes, glycolytic degradation of glucose to lactate and subsequent reconversion of the latter into hexose phosphates and glycogen, occur in the same cell.

INTRODUCTION

The amphibian oocyte has been used as a model system for the study *in vivo* of glucose metabolism and its control, since it is amenable, by microinjection, to the controlled modification of the levels of intermediates which cannot otherwise enter the cell. Labelled glucose (microinjected or present in the incubation medium) is readily incorporated into glycogen and, to a minor extent, released as CO_2 through the pentose phosphate pathway only, since [$6-^{14}C$]glucose does not produce $^{14}CO_2$ [1,2]. All enzymes of glucose utilization are present in oocyte homogenates [1], including phosphofructokinase [3] and fructose-1,6-bisphosphatase [4], previously thought to be absent from these cells [5]. A recent paper from our laboratory [3] reported that a significant amount of carbon from [$U-^{14}C$]glucose was found, after 5 min incubation, in lactate. It was also shown that labelled ATP was formed, after 1 min, from microinjected fructose 6- $[^{32}P]$ phosphate. From these results it was concluded that glycolysis to C_3 compounds is operative in stage VI frog oocytes.

As mentioned above, glycogen is the main end product of glucose metabolism in oocytes, and thus the finding that a substantial part of microinjected glucose is first converted into lactate raises interesting questions about glycogen synthesis in these cells. A parsimonious explanation of these seemingly disparate results is that at least part of the newly synthesized glycogen molecules derive from lactate through the gluconeogenic pathway. This metabolic route has been designated as the ‘indirect’ pathway to distinguish it from the conventional ‘direct’ one (i.e. glucose $\rightarrow$ glucose 6-phosphate $\rightarrow$ glucose 1-phosphate $\rightarrow$ UDP-glucose $\rightarrow$ glycogen). In rats, postprandial glycogen replenishment is mostly brought about through an indirect pathway that requires prior degradation of glucose to C_3 compounds, which are then converted via gluconeogenesis into glucose 6-

phosphate, glucose 1-phosphate, and ultimately into glucosyl units of glycogen. However, the contributions of the direct and indirect pathways to glycogen synthesis are controversial and greatly dependent on the experimental design.

In the research described herein, a variety of approaches were employed to analyse the pathway of glycogen synthesis in frog oocytes, a simple system which is also preferentially glycogenic. For all practical purposes it can be considered as a single cell and therefore free of several recurrent questions about intercellular compartmentation [6]. In this paper we report data obtained from precursor–product relationships, isotopic dilution experiments, measurements of $^3H/^{14}C$ ratios in glycogen derived from specifically labelled glucose molecules, and inhibition of glycogen synthesis by 3-mercaptopicolinate. From the results presented and discussed in this paper, we conclude that, in frog oocytes, glycogen is synthesized preferentially by an indirect pathway involving prior breakdown of glucose to lactate.

EXPERIMENTAL

Materials

Labelled compounds were from The Radiochemical Centre (Amersham). Before the experiment, aliquots were dried under N_2 to remove ethanol and, after the addition of carrier solutions, were resuspended in Barth saline [7] to achieve the desired concentration. HPLC (Dionex CarboPac PA1 column) was used to check for partial hydrolysis and purity of commercial metabolites. Scintillants were from New England Nuclear. Non-radioactive compounds were mostly from Sigma. 3-Mercaptopicolinate was generously given by Dr. H. Werner Hofer (University of Constance, Germany).

‡ To whom correspondence should be addressed.

Animals

Freshly excised stage VI oocytes from the Chilean frog, *Caudiverbera caudiverbera*, obtained from a local dealer and maintained in the laboratory, were used.

Microinjection procedures and metabolic labeling

Microinjection and handling of compounds were as previously described [3]. Briefly, approx. 50 nl of Barth saline [7] containing either radioactive glucose alone or with unlabelled intermediates was injected into groups of 3–5 oocytes, which were incubated at 22 °C under 100 % O₂ and further processed as described below.

Glycogen isolation

Two procedures were employed. (a) At the end of the incubation period, cells were frozen in liquid nitrogen and homogenized in cold 3 % HClO₄. The neutralized HClO₄ extract from a pool of 5–10 oocytes was centrifuged, and 0.2 % Na₂SO₄ and 0.25 mg of carrier glycogen (final concentrations) were added to the supernatant fraction. Glycogen was isolated by repeated precipitation with 50 % ethanol, the resulting pellet was dissolved in warm water and radioactivity was measured (Packard 1600 TR liquid-scintillation analyser). Aliquots of glycogen-free HClO₄ extracts were lyophilized and stored for further analysis. (b) After incubation, each individual oocyte was digested with boiling 30 % KOH, and then 0.07 % Na₂SO₄ and 0.25 mg of carrier glycogen (final concentrations) were added. Glycogen was purified by repeated ethanol precipitation, collected on glass-fibre filters (Whatman GF/C), dried under an IR lamp, and radioactivity was measured (Packard 1600 TR liquid-scintillation analyser).

Separation of metabolic intermediates by ion-exchange chromatography

Separation and identification of metabolic intermediates was as described in [3], except that the CarboPac PA1 column was equilibrated with 90 % solution A (100 mM NaOH) and 10 % solution B (1 M sodium acetate/100 mM NaOH) and eluted at a flow rate of 0.5 ml/min with the following gradient: T₀ = 10 %; T₂₀ = 15 %; T₂₁ = 20 %; T₅₁ = 50 %; T₆₅ = 50 % (subscript numbers are time, in min; percentages are the volume of solution B). Radioactivity measurements were taken every 30 s (Beckman 171 Radioisotope Detector) and processed by the ChromatoGraphics program supplied by Beckman. Labelled intermediates were identified by comparing retention times with those of authentic commercial compounds included in the sample or by incubation of HClO₄ extracts with specific enzymes [3,4].

Measurement of phosphoenolpyruvate carboxykinase activity

Oocytes (about 3 g) were homogenized in 4 ml of 10 mM triethanolamine buffer, pH 7.5, containing 1 mM EDTA, 1 mM dithiothreitol and 0.25 M sucrose. After centrifugation at 105000 g for 1 h, the supernatant fraction was applied to a DEAE-cellulose column equilibrated with the same buffer. The column was washed with 5 vol. of 40 mM KCl in triethanolamine buffer and the enzyme was eluted with 100 mM KCl. Enzyme activity was measured by the coupled malate dehydrogenase method [8]. The reaction mixture contained 50 mM Hepes, pH 7.0, 0.14 mM NADH, 0.1 mM GDP, 0.5 mM phosphoenolpyruvate, 2.2 mM MnCl₂, 50 mM NaHCO₃ and 3 units of malate dehydrogenase in a final volume of 1 ml. The oxidation of

NADH was monitored by measuring the decrease in A₃₄₀ (Hewlett Packard 8452A diode array spectrophotometer). A unit of enzyme activity is defined as the amount of enzyme catalysing the formation of 1 μmol of NAD⁺ under the specified conditions. Inhibition assays with 3-mercaptopicolinate were performed with the partially purified enzyme and 40 μM phosphoenolpyruvate. Mercaptopicolinate was dissolved in water and neutralized to pH 7 with KOH before final dilution immediately before the experiment. The concentration was estimated by titration with 0.5 mM 5,5'-dithiobis-(2-nitrobenzoic acid) at 412 nm.

RESULTS

Kinetics of appearance of radioactive compounds after [¹⁴C]glucose microinjection into frog oocytes

Oocytes microinjected with 0.5 nmol of [U-¹⁴C]glucose were incubated for various times, and the respective HClO₄ extracts were chromatographed (CarboPac column). A typical experiment using short time intervals (5 s, 30 s and 8 min) is depicted in Figure 1. In the first 5 s following injection (the shortest possible time interval between injection and freezing) about 37 % of the

Figure 1 Intermediates of glucose utilization after short-time incubations in frog oocytes microinjected with [U-¹⁴C]glucose

Oocytes were microinjected with saline containing 0.5 nmol of labelled glucose (around 50 000 c.p.m.). After incubation, HClO₄ extracts were chromatographed as described in the Experimental section. Panel (A) shows the chromatogram of the injected substrate. Panels (B), (C) and (D) show the chromatograms corresponding to oocytes incubated for 5 s, 0.5 min and 8 min respectively. Labelled peaks: 1, elution position of glucose; 2, lactate; 3, unidentified compound; 4, glucose 1-phosphate; 5, glucose 6-phosphate; 6, fructose 6-phosphate.

Figure 2 Kinetics of intermediate formation after microinjection of [U- ^{14}C]glucose into frog oocytes

Oocytes were microinjected and processed exactly as described in the legend of Figure 1. Time curves for each intermediate were determined by integrating the areas under the respective peaks of chromatograms of oocyte extracts incubated for the indicated times. For the sake of clarity, curves are shown in two different panels.

label was already present in peak 2, which is mostly lactate, and in another unidentified fraction (Figure 1B). A maximum of about 60% of the total radioactivity present in the HClO_4 extract was incorporated into lactate within 0.5–1 min. Labelling of hexose phosphates was detected several minutes later (Figure 1D). Lactate did not leave the oocyte, since chromatograms of the incubation medium showed traces of labelled glucose only (results not shown). The contribution of the follicular cells to lactate metabolism, although theoretically possible, was considered unlikely, but was not investigated. A time curve for the appearance of radioactivity in several compounds, obtained by integration of the areas under the peaks of the chromatograms, is shown in Figure 2. The lactate peak was greatest between 0.5 and 1 min and diminished rapidly thereafter to reach a plateau at around 8 min. Labelled glycogen began to increase after a lag phase of about 6 min. Hexose phosphates reached a maximum at about 8 min. The burst of lactate production and the shapes of the curves for the other intermediates suggest a precursor–product relationship of lactate with respect to glycogen and its intermediates.

Effect of unlabelled C_3 compounds on the incorporation of ^{14}C from microinjected [1- ^{14}C]glucose into glycogen

If glucose is metabolized to lactate before its incorporation into glycogen, expansion of the pool of unlabelled intermediates would result in dilution of the label incorporated into the polysaccharide. To test this hypothesis, unlabelled triose phosphates or related compounds were co-injected with [1- ^{14}C]glucose (Table 1). A marked decrease in ^{14}C incorporation into glycogen was observed with dihydroxyacetone phosphate,

Table 1 Effect of unlabelled C_3 compounds on the incorporation of [1- ^{14}C]glucose in glycogen by frog oocytes

Oocytes were injected with saline containing 0.4 nmol of [1- ^{14}C]glucose (about 70 000 c.p.m.) and 10.6 nmol of unlabelled compounds as indicated. Three oocytes (in duplicate flasks) were incubated for 30 min. After incubation, each oocyte was individually processed for glycogen isolation as described in the Experimental section. Results are the means $\pm$ S.E.M. of six observations.

Unlabelled compounds co-injected	Fraction of injected label recovered in glycogen
None	0.47 ± 0.056
Dihydroxyacetone phosphate	0.11 ± 0.031
Glyceraldehyde 3-phosphate	0.05 ± 0.009
3-Phosphoglycerate	0.05 ± 0.006
2-Phosphoglycerate	0.39 ± 0.028
Phosphoenolpyruvate	0.16 ± 0.022
Pyruvate	0.42 ± 0.038

Figure 3 Effect of glyceraldehyde 3-phosphate concentration on [1- ^{14}C]glucose incorporation into glycogen by frog oocytes

Oocytes were co-injected with saline containing variable amounts of unlabelled glyceraldehyde 3-phosphate and 0.4 nmol of labelled glucose (about 40 000 c.p.m.). Four oocytes were incubated for 30 min as indicated in the Experimental section. At the end of the incubation time, each oocyte was individually digested with 30% KOH at 100 $^{\circ}\text{C}$, and glycogen was isolated by repeated ethanol precipitation and its radioactivity measured as described. Results are given as means $\pm$ S.E.M. of four individual observations.

glyceraldehyde 3-phosphate, 3-phosphoglycerate or phosphoenolpyruvate. No effect was observed with either 2-phosphoglycerate or pyruvate. The effect of glyceraldehyde 3-phosphate was further characterized. Isotopic dilution by this triose phosphate was the same with either [1- ^{14}C] or [6- ^{14}C]glucose when microinjected or when present in the incubation medium (results not shown). Co-injection of variable amounts of glyceraldehyde 3-phosphate with a fixed amount of glucose (0.4 nmol) showed a dose-dependent decrease in glucose incorporation into glycogen (Figure 3). Glycogen labelling in the presence of unlabelled glyceraldehyde 3-phosphate was about the same (Table 2) irrespective of the radioactive substrate co-injected (glucose, glucose 6-phosphate, glucose 1-phosphate or UDP-glucose).

Glycogen synthesis in oocytes microinjected with doubly labelled glucoses

In order to estimate the relative contributions of pathways other than the direct one, mixtures of [U- ^{14}C]glucose and glucose with

Table 2 Effect of unlabelled glyceraldehyde 3-phosphate on the incorporation of radioactive substrates into glycogen in frog oocytes

Oocytes were injected with saline containing 0.4 nmol of each labelled substrate (about 50 000 c.p.m.) as indicated. Other oocytes were injected with the same solution plus 2.4 nmol of unlabelled glyceraldehyde 3-phosphate (indicated by +). Four oocytes (in duplicate flasks) were incubated for 30 min. After incubation, each oocyte was individually treated as described in the Experimental section. Results are given as the means $\pm$ S.E.M. of eight individual observations.

Substrate	Glyceraldehyde 3-phosphate	Fraction of injected label recovered in glycogen
[U- ¹⁴ C]Glucose	—	0.40 $\pm$ 0.04
	+	0.09 $\pm$ 0.009
[U- ¹⁴ C]Glucose 6-phosphate	—	0.09 $\pm$ 0.003
	+	0.03 $\pm$ 0.003
[U- ¹⁴ C]Glucose 1-phosphate	—	0.34 $\pm$ 0.019
	+	0.09 $\pm$ 0.013
UDP-[6- ³ H]glucose	—	0.29 $\pm$ 0.016
	+	0.14 $\pm$ 0.009

Table 3 Relative ³H/¹⁴C ratios in glycogen from oocytes microinjected with doubly labelled glucoses

Ten oocytes were injected with 50 nl of a mixture containing 0.5 nmol of [U-¹⁴U]glucose and glucose tritiated in a specific position and incubated for 15 or 30 min. At the end of the incubation time, each oocyte was individually digested in hot KOH, glycogen was isolated by repeated ethanol precipitation, and radioactivity was measured. The values are the means $\pm$ S.E.M. of ten individual observations for each experimental condition. Relative ³H/¹⁴C is the ³H/¹⁴C ratio in glycogen divided by the ³H/¹⁴C ratio of the microinjected glucose {values of ³H/¹⁴C (d.p.m./d.p.m.) of the injected glucoses were: [U-¹⁴C,2-³H] = 2.2; [U-¹⁴C,3-³H] = 4.3; [U-¹⁴C,5-³H] = 2.3; [U-¹⁴C,6-³H] = 2.5}. Values in parentheses 100 (1–relative ³H/¹⁴C).

Injected substrate	Relative ³ H/ ¹⁴ C			
	15 min	%	30 min	%
[U- ¹⁴ C,2- ³ H]glucose	0.08 $\pm$ 0.007	(92)	0.07 $\pm$ 0.003	(93)
[U- ¹⁴ C,3- ³ H]glucose	0.73 $\pm$ 0.015	(27)	0.66 $\pm$ 0.011	(34)
[U- ¹⁴ C,5- ³ H]glucose	0.59 $\pm$ 0.020	(41)	0.54 $\pm$ 0.018	(46)
[U- ¹⁴ C,6- ³ H]glucose	0.87 $\pm$ 0.006	(13)	0.87 $\pm$ 0.003	(13)

³H in specific positions were injected into oocytes. The rationale of these experiments [9] was that, if glucose was converted directly into glycogen, the ³H/¹⁴C ratio of the latter would be the same as that of microinjected glucose. Conversely, when glucose is first metabolized to lactate (and other intermediates) which are then used for glycogen synthesis, the ³H/¹⁴C ratio in glycogen should be lower than that of the microinjected glucose. With [2-³H,U-¹⁴C]glucose as substrate (Table 3), extensive detritiation (93 %) of the glucosyl units in glycogen was observed, suggesting that interconversion of glucose 6-phosphate and fructose 6-phosphate is at or near equilibrium in oocytes. This result correlates well with the fact that phosphoglucose isomerase is one of the most abundant carbohydrate-metabolizing enzymes in these cells [1].

Co-injection of [3-³H]- or [5-³H]-glucose and [U-¹⁴C]glucose resulted in 34 % and 46 % detritiation respectively, suggesting that at least one-third of the glucosyl units incorporated into glycogen did not derive from the direct pathway. ³H from positions 3 or 5 of glucose are exchanged with water protons in the isomerization of triose phosphates. It must be noted, however, that ³H from position 5 of glucose can also be lost in the

Figure 4 Inhibition of oocyte phosphoenolpyruvate carboxykinase activity by 3-mercaptopycolinate

The activity of 75 m-units of partially purified enzyme was determined in the presence of increasing concentrations of 3-mercaptopycolinate. Enzyme preparation and activity measurements were as described in the Experimental section. The IC₅₀ value was calculated from the data plotted in the inset. V, velocity of the reaction without inhibitor; v, velocity of the reaction at different inhibitor concentrations.

transaldolase-catalysed reaction. Finally, injection of [6-³H,U-¹⁴C]glucose resulted in 13 % detritiation in glycogen, a result which implies reversibility and cycling of the whole sequence of compounds between glucose and pyruvate and suggests the operation of the phosphoenolpyruvate cycle reactions [10]. The results shown in Tables 1 and 3 indicate that at least one-third of the radioactive glucose reaches the triose phosphate level. Since loss of ³H from C-6 of glucose is only 13 %, this suggests that cycling at the pyruvate level is very low, taking into account that ³H from C-6 may be lost by reactions other than cycling of pyruvate [11].

Effect of 3-mercaptopycolinate on phosphoenolpyruvate carboxykinase activity from oocytes

In order to establish whether glycogen synthesis from glucose involves cycling via pyruvate, the activity of phosphoenolpyruvate carboxykinase was measured. The presence of this enzyme has not been studied previously in frog oocytes. Values of about 0.4 units/g of fresh oocytes were found, which are lower than the levels reported for mammalian liver (1.5 units/g) and kidney (5.3 units/g) [12]. The oocyte enzyme was inhibited by 3-mercaptopycolinate with an IC₅₀ value of 0.47 mM (Figure 4). Inhibition by mercaptopycolinate has been reported for phosphoenolpyruvate carboxykinase from several sources, such as chicken [13], rodents [14], tapeworm [15] and *Trypanosoma* [16].

Effect of 3-mercaptopycolinate on [U-¹⁴C]glucose incorporation into glycogen

If glycogen synthesis proceeds via gluconeogenesis in oocytes, inhibition of phosphoenolpyruvate carboxykinase activity should result in significant blockade of polysaccharide formation from labelled glucose. Indeed, strong inhibition of the incorporation of glucose into glycogen was found in cells co-injected with [U-¹⁴U]glucose and 3-mercaptopycolinate (Figure 5). The compound inhibited the incorporation of [U-¹⁴C]glucose in a dose-dependent manner, with an IC₅₀ value of 0.58 mM (Figure 5), similar to that found *in vitro* for the inhibition of phosphoenolpyruvate carboxykinase activity (Figure 4).

Figure 5 Effect of the amount of injected 3-mercaptopycolinate on the incorporation of carbon from $[U-^{14}C]$ glucose into glycogen by frog oocytes

Oocytes were microinjected with 50 nl of a saline solution containing 0.5 nmol of $[U-^{14}C]$ glucose (about 50 000 c.p.m.). Other oocytes were injected with the same solution containing the indicated amounts of 3-mercaptopycolinate. Groups of 5 oocytes (in triplicate) were incubated for 15 min as indicated in the Experimental section, and each oocyte was then digested with 30% KOH at 100 °C. Glycogen was isolated by repeated ethanol precipitation and radioactivity was measured as described. Results are given as means $\pm$ S.E.M. of 15 individual observations.

DISCUSSION

Traditional views of carbon metabolism in frog oocytes and early amphibian embryos maintain that it is largely glycolytic [17]. Indeed, glycogen is the main product of glucose metabolism in these cells, but the present data support evidence for an indirect pathway of polysaccharide synthesis. This implies prior degradation of glucose to lactate, which is then committed through the gluconeogenic pathway to resynthesize hexose phosphates, which serve as proximate substrates for glycogen synthesis. The kinetics of lactate production and glycogen formation suggest a precursor-product relationship between lactate and the polysaccharide (Figure 2). Lactate production occurred at very low steady-state levels of intermediates, since the corresponding peaks of radioactivity at short incubation times (5–30 s) were not observed. The possibility that the intermediates appear at high transient levels at even shorter times (less than 5 s) was not studied.

Experimental evidence favouring an indirect pathway for glycogen synthesis *in vivo* in liver was first reported by Newgard et al. [9,18] and Kuwajima et al. [19], using isotopic techniques similar to those frequently used in studies of futile cycling in glucose metabolism [10]. The relative contributions of glucose carbon to hepatic glycogen formation by the direct and indirect pathways are greatly dependent on experimental design. In the rat, under physiological refeeding conditions *in vivo*, Kurland and Pilkis [20] found that both pathways were used, each contributing approx. 50% of the carbon appearing in glycogen. However, Huang and Veech [21] found that glycogen synthesis in liver was derived mostly (more than 80%) from the direct pathway. More recently, Shulman et al. [22] found that 50% of carbon in glycogen was via the indirect route following intravenous glucose infusions in overnight-fasted human volunteers. Also, Moore et al. [23] reported that in conscious dogs 43% of glycogen synthesis occurred by the indirect pathway (see [24–26] for reviews).

One of the most widely employed procedures to study the relative contribution of the direct and indirect pathways to liver glycogen formation is the comparison of $^3H/^{14}C$ ratio between glucose specifically labelled with 3H and ^{14}C with those measured in the newly formed glycogen. The lower the relative $^3H/^{14}C$

ratio in glycogen, the greater is the contribution of the indirect pathway. Using this approach, we observed significant detritiation of glucosyl units in glycogen from frog oocytes. With $[U-^{14}C,3-^3H]$ glucose as substrate, the relative $^3H/^{14}C$ ratio in glycogen was 0.66, very similar to that reported by Newgard et al. [9] for fasted rats. It is generally accepted that detritiation of $[3-^3H]$ glucose indicates metabolism through glycolysis. The 12% excess detritiation from $[5-^3H]$ glucose with respect to $[3-^3H]$ glucose observed in oocytes can be attributed to exchange of hydrogen atoms catalysed by transaldolase and correlates well with the low activity of the pentose phosphate cycle previously reported for these cells [1,6]. With $[6-^3H,U-^{14}C]$ glucose, a ratio of 0.87 was found in glycogen. This result is in agreement with values of between 0.8 and 0.9 for mammals reported by several authors (for reviews see [24,27]). However, 3H from C-6 can be lost by other potential mechanisms [11,28], although Katz [29] found these reactions unlikely to occur, at least in liver.

Although of great value, results obtained with tritiated substrates should be interpreted with caution [10]. For example, underestimation of detritiation ratios may result from dilution of labelled gluconeogenic intermediates in indirect pathways where glucose carbons participate in more metabolite pools than when glucose is metabolized by the direct route.

Our proposition (based in the studies of relative $^3H/^{14}C$ ratios) that an indirect route for glycogen synthesis operates in oocytes was reinforced by the marked dilution of the label in glycogen when radioactive glucose was co-injected with unlabelled C_3 compounds. The concentrations of these metabolites in *Caudi verbera* oocytes are unknown. Values of 100 μM for pyruvate and 20 μM or less for 3-phosphoglycerate have been reported for *Xenopus* oocytes [5]. In resting rat muscle the concentrations of these intermediates are around 100 μM , with a highest value of 380 μM for pyruvate and the lowest value of 20 μM for 2-phosphoglycerate [30]. In isotopic dilution experiments (Table 2), 10.6 nmol of unlabelled compounds was injected, which, assuming a cell volume of 3 μl and free diffusion within the oocyte, gives a cellular concentration of 3.5 mM, a value which is more than 30 times the estimated concentration reported for rat cells. Thus, if microinjected metabolites are able to mix with the internal pool of labelled glycolytic intermediates, an isotopic dilution effect would be expected. Moreover, in the case of glyceraldehyde 3-phosphate, the maximal effect is obtained at a cellular concentration of 0.66 mM (Figure 3), which is 6-fold higher than the reported internal concentration. Notably, 50% of the effect was obtained at a concentration almost identical with the concentration of injected glucose (0.4 nmol), which further supports the suggestion of isotopic dilution. Since radioactive triose phosphates are not commercially available, the obvious experiment of injecting these compounds into oocytes was not performed.

The lack of isotopic dilution by unlabelled 2-phosphoglycerate or pyruvate is surprising. One explanation with respect to 2-phosphoglycerate would be the existence of a direct metabolite transfer by enzyme-enzyme interactions. In support of this suggestion, the cellular concentration of 2-phosphoglycerate is the lowest of all glycolytic intermediates, and virtually all 1,3-bisphosphoglycerate is bound to phosphoglycerate kinase, the aqueous concentration of the compound being exceedingly low [30]. We propose an alternative explanation for the lack of effect of pyruvate. When labelled pyruvate or lactate was injected, or supplied in the medium, to oocytes, no radioactivity was incorporated into glycogen [6], even in the presence of unlabelled glucose. Nevertheless, high production of $^{14}CO_2$ was observed. These results suggest that exogenous pyruvate (or lactate) cannot gain access to a gluconeogenic compartment, but enters the citric

acid cycle instead. Experiments are being performed to study this possibility.

That the indirect pathway accounts for substantial glycogen synthesis in oocytes is shown by the results of inhibition studies of phosphoenolpyruvate carboxykinase by 3-mercaptopicolinate, a classical gluconeogenic inhibitor [14,31]. Co-injection of labelled glucose and 1 mM mercaptopicolinate (intracellular concentration) diminishes label incorporation into glycogen by 80 %, a result which suggests that a large part of the radioactivity derived from glucose reaches pyruvate before being incorporated into the polysaccharide. The essentiality of an active gluconeogenic pathway for efficient hepatic glycogen synthesis from exogenous glucose, or C_3 precursors, has been demonstrated in several cases by the use of 3-mercaptopicolinate [18,31–35].

Some authors have suggested an extrahepatic origin for C_3 precursors or separation of glycolysis and gluconeogenesis in different hepatocyte populations (for reviews see [25,26]). However, recent reports suggest that intrahepatic glycolysis is the source of most of the C_3 substrates for the indirect pathway [23]. Moreover, glucose recycling via pyruvate in isolated hepatocytes [28] requires both gluconeogenic and glycolytic capacities in the same cell. Besides the overwhelming evidence that glycogen deposition in rat liver is mainly a gluconeogenic process, most workers have stressed the fact that the indirect pathway is hepatic only. Our results indicate that the indirect route is operative in frog oocytes, and a recent paper [34] reports a similar conclusion for human astrocytes. Moreover, we have unambiguously shown that both the glycolytic breakdown of glucose to lactate, and the subsequent reconversion of the latter through gluconeogenesis to glucosyl units in glycogen, take place in the same cell.

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