

Developmentally related changes in the metabolism of glucose and glutamine by cattle embryos produced and co-cultured *in vitro*

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Summary. The metabolism of radiolabelled glucose and glutamine was measured in individual cattle embryos produced by *in vitro* maturation and fertilization of oocytes, and culture with bovine oviductal epithelial cells. Metabolism of glucose through the pentose-phosphate pathway increased almost 15 times and the total metabolism of glucose 30 times, during development from the two-cell to the expanded blastocyst stage. The first marked increase in glucose metabolism did not occur until between the eight- and 16-cell stages, the time of activation of the embryonic genome. Conversely, the metabolism of glutamine was high in two- and four-cell embryos and then decreased to reach a minimum at the compacted morula to blastocyst stage, possibly because of degradation of maternally derived enzymes. Blastocyst expansion was accompanied by significant increases in the metabolism of glucose and glutamine, presumably reflecting the increased energy demands of $\text{Na}^+ - \text{K}^+$ ATPase necessary for formation and maintenance of the blastocoel.

Keywords: cattle; embryo; energy metabolism; development; glutamine

Introduction

Numerous studies have shown significant changes in the uptake, metabolism and incorporation of a variety of energy substrates and amino acids during the early development of pre-implantation mouse and rabbit embryos (see Rieger, 1984; Kaye, 1986). In contrast, little information is available about developmental changes in the metabolic activity of embryos of domestic species, although glucose metabolism has been shown to increase during early development in pig (Flood & Wiebold, 1988) and sheep (Thompson *et al.*, 1991) embryos. In embryos collected from superovulated heifers, 7 days after insemination, measurable amounts of glucose, glutamine and pyruvate were metabolized by blastocysts after 24 h in culture (Rieger & Guay, 1988), and glucose and glutamine metabolism increased between the morula and expanded blastocyst stages (Tiffin *et al.*, 1991). Glucose metabolism of embryos collected from superovulated heifers increases from the 16-cell stage onward (Javed & Wright, 1991). However, in general, the difficulty and expense of collecting embryos at earlier stages from cattle by surgery or slaughter precluded the study of their metabolic patterns until the advent of reliable techniques for embryo production *in vitro* (e.g. Xu *et al.*, 1991).

In this study, we measured the metabolism of radiolabelled glucose and glutamine by cattle embryos, from the early cleavage stages, through the time of activation of the embryonic genome at the eight- to 16-cell stage (Telford *et al.*, 1990), and up to differentiation and expansion of the blastocyst *in vitro*. Among a variety of possible energy substrates, glucose and glutamine are of particular interest. In hamsters and strains of mice that exhibit the two-cell block to development *in vitro*, glutamine is necessary for early embryonic development (Carney & Bavister, 1987; Chatot *et al.*, 1989; Bavister & Arlotto, 1990), whereas glucose is detrimental (Schini & Bavister, 1988;

Chatot *et al.*, 1989). Glucose or glutamine, alone or in combination, support the development of one- and two-cell pig embryos to the blastocyst stage (Petters *et al.*, 1990). Glucose concentrations of $> 1.5 \text{ mmol l}^{-1}$ are detrimental to the development of one- and two-cell sheep embryos to the blastocyst stage (J. G. E. Thompson, pers. commun.).

In this study, the production of $^3\text{H}_2\text{O}$ from D-[5- ^3H]glucose was used as a measure of total glucose metabolism. The production of $^{14}\text{CO}_2$ from D-[1- ^{14}C]glucose and D-[6- ^{14}C]glucose was used to determine the relative amounts of glucose metabolized by the pentose-phosphate pathway and the Krebs cycle. The oxidation of glutamine is exclusively mitochondrial and therefore the production of $^{14}\text{CO}_2$ from L-[^{14}C (U)]glutamine and of $^3\text{H}_2\text{O}$ from L-[3,4- ^3H (N)]glutamine were used as measures of the activity of the Krebs cycle (see Tiffin *et al.*, 1991).

Materials and Methods

In three experiments, the metabolism of radiolabelled glucose and glutamine was measured in various combinations in individual embryos prepared from separate batches of oocytes.

Oocyte collection and *in vitro* fertilization

For each experiment, 200–300 oocytes were recovered from 20–25 ovaries obtained from a local abattoir, as previously described (Xu *et al.*, 1992). Cumulus–oocyte complexes, with at least 2–3 compact layers of granulosa cells, were cultured in groups of 10–15 in 50 μl microdrops of Ham's F-10 medium (Gibco, Grand Island, NY, USA) supplemented with 20% (v/v) oestrous cow serum, 0.4 mmol L-glutamine l^{-1} (Sigma Chemical Co., St Louis, MO, USA), 0.2 mmol sodium pyruvate l^{-1} (Sigma), 100 u penicillin ml^{-1} and 100 μg streptomycin ml^{-1} (Gibco). The microdrops were overlaid with 1.0 ml silicone oil (Aldrich Chemical Co., Milwaukee, WI, USA), previously sterilized by filtration (0.22 μm) and equilibrated with the culture medium by mixing equal volumes and incubating for 24–48 h. After culturing for 24–26 h in a humidified atmosphere of 5% CO_2 in air at 39°C, the oocytes were fertilized as previously described (Greve *et al.*, 1987).

Embryo co-culture

Eighteen hours after addition of the spermatozoa to the oocytes, approximately 25 presumptive zygotes were placed in 50- μl microdrops of Ménéz B2 medium (IMV International, Minneapolis, MN, USA), supplemented with 10% (v/v) oestrous cow serum and bovine oviductal epithelial cells that were prepared as described by Xu *et al.* (1992). The microdrops were overlaid with 1.0 ml silicone oil and incubated in a humidified atmosphere of 5% CO_2 in air at 39°C.

Just before the metabolic measurements, embryos were removed from the culture droplets, divested of any adhering cumulus cells using a micropipette (inner diameter 100–120 μm), passed through four washes of B2 supplemented with 20 mmol *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid l^{-1} (Hepes, Sigma) and cultured in the last wash for 2 h at 39°C under 5% CO_2 in air (pH 7.4). Relative to the time of insemination, two- and four-cell embryos were obtained at 40 h, eight-cell at 66 h, 16-cell at 90 h, compact morulae at 138 h and blastocysts and expanded blastocysts at 162 h.

Radiolabelled substrates

D-[1- ^{14}C]glucose (sp. act. 56.6 mCi mmol^{-1}), D-[6- ^{14}C]glucose (55.8 mCi mmol^{-1}), L-[^{14}C (U)]glutamine (237.6 mCi mmol^{-1}), and L-[3,4- ^3H (N)]glutamine (52.2 Ci mmol^{-1}) were purchased from New England Nuclear, Boston, USA. D-[5- ^3H]glucose (21.1 Ci mmol^{-1}) was purchased from Amersham International, Amersham, UK. Mixtures of one ^3H -labelled and one ^{14}C -labelled substrate were dried under nitrogen, taken up to B2–Hepes medium to give nominal concentrations of 0.25 $\mu\text{Ci } \mu\text{l}^{-1}$ for each labelled substrate. In Expt 1, D-[1- ^{14}C]glucose was mixed with D-[5- ^3H]glucose. In Expts 2 and 3, two different combinations of labelled substrates were used in parallel: D-[1- ^{14}C]glucose with L-[3,4- ^3H (N)]glutamine for Expt 2a, L-[^{14}C (U)]glutamine with D-[5- ^3H]glucose for Expt 2b, D-[1- ^{14}C]glucose with D-[5- ^3H]glucose for Expt 3a and D-[6- ^{14}C]glucose with L-[3,4- ^3H (N)]glutamine for Expt 3b. These solutions were stored at 4°C for the duration of each experiment and incubated at 39°C under 5% CO_2 in air for 2 h before each measurement period.

Metabolic measurements

A hanging-drop technique, similar to that described by O'Fallon & Wright (1986), was modified to allow the use of the bicarbonate-buffered B2 medium (Fig. 1). Individual embryos were taken up in 2 μl of the final wash and placed

in the cap of a sterile 2.5 ml polypropylene screw-cap microtube (T. M. Sarstedt Inc., St Laurent, Québec, Canada) with 2 μl of the appropriate mixture of radiolabelled substrates (total culture volume of 4 μl). The cap was quickly fitted onto the tube, which had been loaded with 1.5 ml of NaHCO_3 (25 mmol l^{-1}) equilibrated with a gas mixture of 5% O_2 , 5% CO_2 and 90% N_2 . Two or three sham preparations, containing all reagents but no embryo, were included for each mixture of labelled substrates in each measurement period. These served to control for all nonspecific counts due to machine background, chemiluminescence, bacterial contamination and spontaneous breakdown of the labelled substrate. Two to five control embryos were left in the last wash of B2-Hepes whilst the measured embryos were in the measurement apparatus, to evaluate the effects of the procedure on subsequent development.

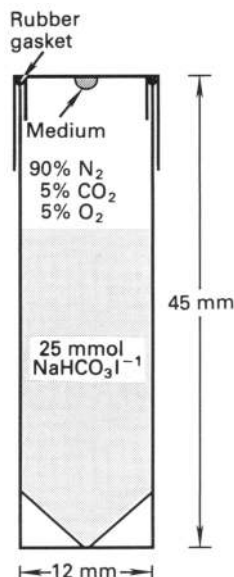


Fig. 1. Diagram of the hanging-drop apparatus used for the measurement of metabolic activity of individual bovine embryos. The 4 μl droplet of Ménézo B2 medium supplemented with 20 mmol Hepes l^{-1} contained one embryo with one ^{14}C - and one ^3H -labelled substrate. The $^{14}\text{CO}_2$ and $^3\text{H}_2\text{O}$ produced during the 3 h of incubation at 39°C exchanged with the bicarbonate and water in the 1.5 ml sodium bicarbonate in the bottom of the vial.

At the end of the 3 h culture period, the caps were removed and the bicarbonate was quickly poured into 20 ml scintillation vials containing 200 μl of NaOH (0.1 mol l^{-1}), to convert the dissolved CO_2 and bicarbonate into carbonate. After being capped and held at 4°C for 16 h, 15 ml of scintillation fluid (HP/b, Beckman Instruments, Fullerton, CA, USA) was added and radioactivity in the vials was measured for 5 min in a liquid scintillation counter, programmed for automatic dual-label correction to disintegrations per minute (d.p.m.). The total d.p.m. of labelled substrate was determined by mixing 2 μl of the labelled substrate solution with 1.5 ml of NaHCO_3 (25 mmol l^{-1}) and counting in the same way.

The embryos were removed from the microtube caps at the end of the 3 h culture period and washed in B2-Hepes. The measured and control embryos were returned to separate culture droplets (containing oviductal cells) and evaluated for development at 10 days after insemination.

Estimation of product recovery

Known amounts of $\text{NaH}^{14}\text{CO}_3$ and $^3\text{H}_2\text{O}$ (New England Nuclear) were taken up in 4 μl droplets of B2-Hepes and cultured for 0–3 h, as for the embryos. At the end of each period, the bicarbonate in the tube was poured into scintillation tubes for measurement of radioactivity as described. The recovery efficiency was expressed as the proportion of radioactivity in the bicarbonate solution.

Calculation of substrate metabolism

The amount of each substrate metabolized by each embryo was calculated as previously (Tiffin *et al.*, 1991). For each labelled substrate, the mean d.p.m. for the sham preparations was subtracted from the d.p.m. for each embryo.

The difference was divided by the total d.p.m. of labelled substrate added, and multiplied by the total quantity of substrate in 4 μ l of medium and by the appropriate recovery correction factor.

Statistical analysis

The effect of developmental stage on the metabolism of each substrate in each experiment was evaluated by one-way analysis of variance of $\log(y + 1)$, where y = the measured metabolism. The log-transformed data for the metabolism of D-[1- 14 C]glucose, D-[5- 3 H]glucose and glutamine were combined across experiments and analysed by two-way analysis of variance (experiment X stage). The log-transformed developmental stage means were compared by Duncan's multiple-range tests. The distributions of final development for the measured and control embryos (combined across all experiments) were compared by χ^2 analysis.

Results

The recovery of $^3\text{H}_2\text{O}$ and $^{14}\text{CO}_2$ increased rapidly up to 60 min and then more slowly, to over 90% by 120 min (Fig. 2). During the 3 h culture period, 77.3 and 70.7% of the total amounts of $^3\text{H}_2\text{O}$ and $^{14}\text{CO}_2$, respectively, were recovered. The metabolic measurements presented therefore include a correction factor of 1.29 (100/77.3) for the ^3H -labelled substrates and 1.41 (100/70.7) for the ^{14}C -labelled substrates.

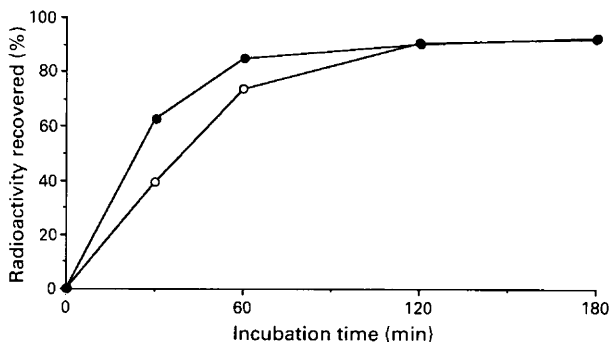


Fig. 2. Recovery of $^3\text{H}_2\text{O}$ (●) and $^{14}\text{CO}_2$ (○) in 1.5 ml of 25 mmol $\text{NaHCO}_3 \text{ l}^{-1}$ in the bottom of the 2.5 ml vial following placement of $^3\text{H}_2\text{O}$ and $\text{NaH}^{14}\text{CO}_3$ in 4 μ l culture medium as a hanging drop in the vial cap. Each point represents the mean of five determinations; SEM too small to be shown.

There was a highly significant effect of stage of development ($P \leq 0.0003$) on the metabolism of all substrates in all experiments (Tables 1, 2 and 3).

The pattern of metabolism of D-[1- ^{14}C]glucose was consistent in all three experiments (1, 2a and 3a): a slight increase from the two-cell to eight-cell stage, a marked increase between the eight- and 16-cell stages and then a continued increase to the expanded blastocyst stage. The metabolism of D-[5- ^3H]glucose (Expts 1, 2b and 3a) was several times greater than that of D-[1- ^{14}C]glucose at every developmental stage. The general pattern of increase in the metabolism of the two substrates was similar, except that the first marked rise for D-[5- ^3H]glucose occurred between the 16-cell and compacted morula stages, one cleavage stage later than for D-[1- ^{14}C]glucose. The metabolism of D-[6- ^{14}C]glucose (Expt 3b) was low compared with the other two labelled forms of glucose at every developmental stage and there was no consistent pattern.

The metabolism of L-[3,4- $^3\text{H}(N)$]glutamine (Expts 2a and 3b) and L-[$^{14}\text{C}(U)$]glutamine (Expt 2b) was high at the two- and four-cell stages and then decreased steadily to reach a minimum at the compacted morula or blastocyst stage, before increasing again at the expanded blastocyst stage.

Two-way analysis of the combined results for the metabolism of D-[1- ^{14}C]glucose, D-[5- ^3H]glucose, and the two labelled forms of glutamine (Table 4) indicated a significant effect of

Table 1. Metabolism of glucose (pmol per embryo per 3 h) by individual bovine embryos (Expt 1)

Stage	Number of embryos	Labelled substrate	
		[1- ¹⁴ C]glucose	[5- ³ H]glucose
2-cell	4	0.12 ± 0.07 ^a	0.68 ± 0.37 ^{ab}
4-cell	3	0.29 ± 0.22 ^a	2.93 ± 0.40 ^c
8-cell	5	0.88 ± 0.43 ^a	0.04 ± 0.04 ^a
16-cell	5	2.22 ± 0.36 ^b	1.92 ± 0.66 ^{bc}
Compacted morula	5	2.65 ± 0.45 ^b	5.81 ± 2.80 ^c
Blastocyst	7	6.68 ± 1.03 ^c	29.30 ± 3.23 ^d
Expanded blastocyst	1	9.02 ^c	65.10 ^d

All values are means ± SEM.

^{a,b,c,d}Within columns, values with different superscripts are significantly different ($P \leq 0.05$).

Table 2. Metabolism of glucose and glutamine (pmol per embryo per 3 h) by individual bovine embryos

Stage	Expt 2a			Expt 2b		
	No. of embryos	Labelled substrate		No. of embryos	Labelled substrate	
		[1- ¹⁴ C]-glucose	[3,4- ³ H]-glutamine		[¹⁴ C(U)]-glutamine	[5- ³ H]-glucose
2-cell	5	0.49 ± 0.23 ^a	6.88 ± 1.01 ^{ab}	5	5.48 ± 0.48 ^a	0.95 ± 0.41 ^a
4-cell	5	0.28 ± 0.13 ^a	10.02 ± 1.74 ^a	5	5.08 ± 0.61 ^a	0.81 ± 0.45 ^a
8-cell	5	0.81 ± 0.20 ^a	5.20 ± 1.19 ^{abc}	5	2.80 ± 0.49 ^b	0.20 ± 0.21 ^a
16-cell	5	1.90 ± 0.24 ^b	2.38 ± 0.58 ^{cd}	5	2.18 ± 0.33 ^{bc}	1.12 ± 0.75 ^a
Compacted morula	5	2.28 ± 0.24 ^b	1.57 ± 0.11 ^d	5	1.62 ± 0.27 ^{bcd}	9.46 ± 1.99 ^b
Blastocyst	4	2.35 ± 0.71 ^b	2.99 ± 0.90 ^{cd}	4	1.08 ± 0.08 ^d	19.70 ± 2.42 ^{bc}
Expanded blastocyst	1	5.63 ^c	4.11 ^b	1	1.38 ^d	37.40 ^c

All values are means ± SEM.

^{a,b,c,d}Within columns, values with different superscripts are significantly different ($P \leq 0.05$).

experiment ($P \leq 0.03$) and stage of development ($P \leq 0.001$) in all three cases. There was also a significant effect of interaction of experiment and stage for D-[1-¹⁴C]glucose ($P \leq 0.02$) and D-[5-³H]glucose ($P \leq 0.0002$), but not for glutamine ($P > 0.30$).

For the measured and control embryos combined, significantly fewer embryos taken at the two-cell (16.7%) or four-cell (28.1%) stage developed to the blastocyst stage, compared with those taken at the eight-cell (65.7%), 16-cell (70.6%) or compacted morula (71.4%) stage (Fig. 3). Compared with the controls, the subsequent development of embryos measured at the two- and four-cell stages was significantly impaired ($P \leq 0.03$), although 67% of the embryos measured at those two stages underwent at least two more cell cycles. The procedure had no statistically significant effect on the development of embryos measured at later stages ($P > 0.15$).

Discussion

The results clearly demonstrate that the hanging-drop technique can be used to measure the metabolic activity of individual cattle embryos in a bicarbonate-buffered complete medium that requires

Table 3. Metabolism of glucose and glutamine (pmol per embryo per 3 h) by individual bovine embryos

Stage	Expt 3a			Expt 3b		
	No. of embryos	Labelled substrate		No. of embryos	Labelled substrate	
		[1- ¹⁴ C]-glucose	[5- ³ H]-glucose		[6- ¹⁴ C]-glucose	[3,4- ³ H]-glutamine
2-cell	5	0.54 ± 0.09 ^a	1.71 ± 0.83 ^a	5	0.00 ± 0.00 ^a	7.80 ± 1.23 ^a
4-cell	5	0.73 ± 0.14 ^a	5.11 ± 0.60 ^b	5	0.19 ± 0.10 ^{ab}	6.54 ± 0.91 ^{ab}
8-cell	5	1.24 ± 0.46 ^a	4.26 ± 0.79 ^{ab}	5	0.53 ± 0.15 ^c	3.87 ± 0.37 ^{bc}
16-cell	5	2.39 ± 0.46 ^b	3.87 ± 1.19 ^{ab}	5	0.02 ± 0.02 ^a	3.32 ± 1.15 ^{cd}
Compacted morula	5	2.62 ± 0.32 ^b	7.98 ± 3.67 ^b	5	0.37 ± 0.08 ^{bc}	2.00 ± 0.32 ^{cd}
Blastocyst	4	2.85 ± 0.47 ^b	18.80 ± 4.56 ^c	6	0.13 ± 0.06 ^{ab}	1.70 ± 0.43 ^d
Expanded blastocyst	2	4.40 ± 2.00 ^b	22.90 ± 6.47 ^c	3	0.36 ± 0.20 ^{bc}	6.57 ± 2.17 ^{ab}

All values are means ± SEM.

^{a,b,c,d}Within columns, values with different superscripts are significantly different ($P \leq 0.05$).

Table 4. Combined data from Expts 1, 2 and 3 for the metabolism of glucose and glutamine (pmol per embryo per 3 h) by individual bovine embryos

Stage	Labelled substrate		
	[1- ¹⁴ C]glucose	[5- ³ H]glucose	Glutamine
2-cell	0.40 ± 0.10 ^a (14)	1.14 ± 0.34 ^a (14)	6.72 ± 0.57 ^a (15)
4-cell	0.46 ± 0.10 ^{ab} (13)	2.95 ± 0.63 ^b (13)	7.21 ± 0.84 ^a (15)
8-cell	0.98 ± 0.21 ^b (15)	1.50 ± 0.58 ^a (15)	3.96 ± 0.49 ^b (15)
16-cell	2.16 ± 0.20 ^c (15)	2.30 ± 0.57 ^{ab} (15)	2.63 ± 0.43 ^c (15)
Compacted morula	2.52 ± 0.19 ^c (15)	7.75 ± 1.60 ^c (15)	1.74 ± 0.15 ^c (14)
Blastocyst	4.50 ± 0.74 ^d (15)	23.90 ± 2.33 ^d (15)	1.89 ± 0.36 ^c (14)
Expanded blastocyst	5.86 ± 1.36 ^e (4)	37.00 ± 10.3 ^d (4)	5.04 ± 1.57 ^b (5)

All values are means ± SEM.

The number of values represented within each mean are shown in parentheses.

^{a,b,c,d}Within columns, values with different superscripts are significantly different ($P \leq 0.05$).

an atmospheric concentration of 5% CO₂. Although the medium was supplemented with 20 mmol Hepes l⁻¹, this had only a limited buffering effect because, when exposed to air, the pH of the 4 µl droplets increased markedly within 10 min, judging by the colour change of the indicator (phenol red). Conversely, there was no noticeable colour change in the droplets over the 3 h incubation period in the measurement apparatus.

After return to culture, the development of embryos taken at the two- and four-cell stages was poor compared with those taken at later stages. This probably reflects a selection bias, in that embryos taken at very early stages would include those destined to cease development before the eight-cell stage. The metabolic measurement procedure also had a deleterious effect on subsequent development, but only at the very early cleavage stages. This suggests that embryos at the very early cleavage stage are more sensitive to manipulation, but the reasons are unknown. Gardner *et al.* (1989) have suggested that the bias due to nonviable embryos must be considered when comparing absolute uptakes of substrates at different stages of embryonic development. In the present study, although most embryos developed to some degree after the measurement procedure, the results clearly include those of nonviable embryos and therefore reflect the metabolic activity of the entire population, rather than just that of viable embryos.

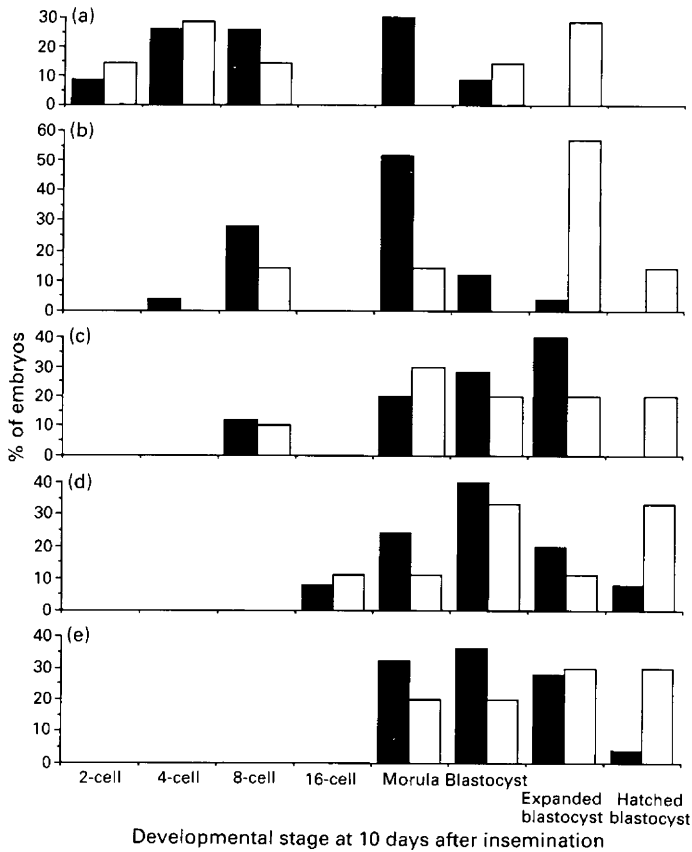


Fig. 3. Proportional distributions for the final development of bovine control embryos (□) and those subjected to the metabolic measurement procedure (■), after return to co-culture and evaluation at 10 days after insemination for embryos taken at the (a) 2-cell, (b) 4-cell, (c) 8-cell, (d) 16-cell and (e) compacted morula stages.

Although there were statistically significant changes in the production of $^{14}\text{CO}_2$ from D-[6- ^{14}C]glucose during development of the embryos, the measurements were only slightly above the sensitivity of the technique. Consequently, we cannot be confident that the apparent oscillations in the metabolism of this substrate are of any physiological significance. However, it is clear that the metabolism of D-[6- ^{14}C]glucose was uniformly lower throughout development than the metabolism of the other two labelled forms of glucose. This suggests that the Krebs cycle plays a limited role, if any, in the metabolism of glucose at all the stages measured and consequently that the release of $^{14}\text{CO}_2$ from D-[1- ^{14}C]glucose was entirely due to processing through the pentose-phosphate pathway (see Tiffin *et al.*, 1991). We have previously observed this phenomenon in cattle blastocysts collected from superovulated animals and concluded that it results from a lack, or inhibition, of pyruvate kinase, a major regulatory enzyme in glycolysis (Rieger & Guay, 1988).

The overall increase in the production of $^3\text{H}_2\text{O}$ from D-[5- ^3H]glucose and of $^{14}\text{CO}_2$ from D-[1- ^{14}C]glucose with development to the expanded blastocyst stage resembles that reported for the metabolism of radiolabelled glucose by mouse (O'Fallon & Wright, 1986; Wales, 1986), pig (Flood & Wiebold, 1988), sheep (Thompson *et al.*, 1991), human (Wales *et al.*, 1987) and *in-vitro*-produced cattle (Javed & Wright, 1991) embryos and resembles the changes in total uptake of glucose with development of mouse (Gardner & Leese, 1988) and human (Gott *et al.*, 1990) embryos. However, the first marked increase in glucose metabolism in the cattle embryos in this study occurred between

the eight- and 16-cell stages, whereas the first marked increase occurs between the four- and eight-cell stages in pig (Flood & Wiebold, 1988) and human (Wales *et al.*, 1987) embryos, and between the two- and eight-cell stages in sheep embryos (Thompson *et al.*, 1991). Javed & Wright (1991) reported a similar pattern of metabolism of glucose by embryos collected from superovulated heifers, except that the first significant increase in the metabolism of D-[1-¹⁴C]glucose and D-[5-³H]glucose occurred between the 16-cell and morula stages. For pig, human, sheep and cattle embryos, the first marked increase in glucose metabolism roughly coincides with the time of activation of the embryonic genome (see Telford *et al.*, 1990). This may suggest that, at least for these four species, the onset of glucose metabolism in the early embryo is related to the synthesis of one or more key glycolytic enzymes. However, the changes in glucose metabolism during development may also be due to changes in allosteric regulation of enzyme activity. For example, the decrease in glutamine metabolism between the four-cell and compacted morula stages may have resulted in a reduced cellular ATP concentration which would reduce the inhibition of phosphofructokinase, and consequently permit increased anaerobic glucose metabolism (see Voet & Voet, 1990).

The timing of changes in glucose metabolism during development of mouse embryos appears to be an exception because, although hexokinase activity increases more than three times between the one- and two-cell stages (Hooper & Leese, 1989), coinciding with activation of the embryonic genome (see Telford *et al.*, 1990), the first marked increase in glucose metabolism does not appear until after the eight-cell stage (O'Fallon & Wright, 1986; Wales, 1986). This delay in the metabolism of glucose reflects the block to glycolysis (Brinster, 1965) which results from an inhibition of phosphofructokinase (Barbehenn *et al.*, 1974, 1978).

The significant increases in the metabolism of D-[5-³H]glucose and D-[1-¹⁴C]glucose between the compacted morula and expanded blastocyst stages were qualitatively and quantitatively similar to our previous measurements of the metabolism of these substrates in embryos collected from superovulated heifers (Tiffin *et al.*, 1991). In rabbit and mouse embryos, the formation of the blastocoele is associated with significant increases in the synthesis and activity of Na⁺-K⁺ ATPase (see Benos & Balaban, 1990), and the increase in glucose metabolism during blastulation in cattle embryos probably also results from the energy demands of this ion pump.

The pattern of change in metabolism of glutamine by cattle embryos in this study was different from the pattern of glucose metabolism. The enzymes responsible for the high rate of glutamine metabolism at the two- and four-cell stages must have been of maternal origin and the significant decrease at the eight-cell stage almost certainly resulted from degradation of the maternally derived enzymes or mRNA, or both. Although the continued decrease of glutamine metabolism through the 16-cell and compacted morula stages was probably due to further degradation of the maternally derived products, it may also have been related to the concurrent increase in glucose metabolism.

In mouse embryos, glutamine uptake increases steadily with development to the blastocyst stage (Brinster, 1971; Gardner *et al.*, 1989; Chatot *et al.*, 1990), as does the uptake of other amino acids (Epstein & Smith, 1973). Chatot *et al.* (1990) also measured glutamine metabolism in mouse embryos that had developed *in vivo* or had been produced by *in vitro* fertilization and culture. Glutamine metabolism by *in vivo* embryos increased from the one-cell to two-cell stage, decreased at the eight-cell stage, and then increased to a maximum at the blastocyst stage. In *in vitro* embryos, glutamine metabolism increased from the two-cell to four-cell stages and then again at the morula stage. In the presence of glucose, glutamine metabolism was reduced in *in vivo* eight-cell embryos and blastocysts and in *in vitro* morulae. It is difficult to draw any direct parallels between the patterns of glutamine metabolism by cattle embryos in the present study and the observations of Chatot *et al.* (1990) in mouse embryos, except that measurable amounts of glutamine were metabolized at all stages in both studies, reaching a maximum at the expanded blastocyst stage in cattle embryos and at the blastocyst stage in mouse embryos.

The significant increase in glutamine metabolism between the compacted morula and expanded blastocyst stages in the present study was similar to that measured in embryos collected from superovulated heifers (Tiffin *et al.*, 1991) and, like glucose metabolism, was probably also related to

the energy demands of $\text{Na}^+ - \text{K}^+$ ATPase. This contention is supported by the fact that, in horse embryos, glucose and glutamine metabolism are proportional to blastocyst size (Rieger *et al.*, 1987, 1989, 1991). From the relationship between glucose metabolism and oxygen consumption, Robinson & Benos (1991) concluded that the oxidative metabolism of glucose is probably not the major pathway by which the rabbit blastocyst obtains its energy. Their conclusion is consistent with our observation that the Krebs cycle plays a limited role in the metabolism of glucose by cattle embryos. Robinson & Benos (1991) speculate that neutral glyceride pools are mobilized to meet the increased energy demands of blastulation, but our results suggest that glutamine metabolism is an equally good candidate for this function, at least in cattle blastocysts.

In general, the rate of metabolism of glucose and glutamine is high in rapidly dividing cells (Newsholme *et al.*, 1985). In cultured human fibroblasts, there is a reciprocal relationship between glucose and glutamine use; glucose metabolism is inhibited by increasing glutamine concentration in the medium, and vice versa (Zielke *et al.*, 1978). However, such a reciprocal relationship could not have been responsible for the changes in metabolism of glucose and glutamine that we observed during development of cattle embryos, because these substrates were used at the same concentrations for all stages studied. Clearly then, the changes can be related to, and may be necessary for, development. This conclusion is supported by comparison of our observations with the known effects of glucose and glutamine on the development *in vitro* of hamster embryos and embryos of strains of mice that exhibit the two-cell block. During early cleavage stages, glutamine favours the development of hamster (Carney & Bavister, 1987; Bavister & Arlotta, 1990) and mouse (Chatot *et al.*, 1989) embryos, and its metabolism by cattle embryos was high. Conversely, glucose is detrimental to the development of early cleavage-stage hamster (Schini & Bavister, 1988) and mouse (Chatot *et al.*, 1989) embryos, and its metabolism by cattle embryos was low.

The relative importance of glucose and glutamine to later embryo development is less clear. Glucose is required for the development of mouse embryos beyond the three- to four-cell stage (Chatot *et al.*, 1989). The development of eight-cell hamster embryos to the blastocyst stage is inhibited by glucose (Seshagiri & Bavister, 1989a, b), but supported by glutamine in the absence of any other energy substrate (Bavister, 1987).

The pattern of glucose metabolism by cattle embryos is also consistent with the effects of glucose on development of cattle embryos *in vitro*. When present up to the eight-cell stage, glucose is detrimental to the subsequent development of cattle embryos cultured with oviductal cells (Ellington *et al.*, 1989). Conversely, for cattle embryos cultured with their adherent cumulus cells, the addition of glucose at 72 h after insemination (when the eight-cell stage would be expected) improved development to the blastocyst stage (Robl *et al.*, 1991). However, it is impossible to know whether the effects of glucose were exerted directly on the embryos, or were mediated by effects on the accompanying cells in these two-culture studies.

Significant changes occur in the metabolic activity of cattle embryos during development to the expanded blastocyst stage in culture with bovine oviductal cells. The increased metabolism of glucose during development of cattle embryos is consistent with the patterns reported in other species, the first significant increase occurring coincident with the activation of the embryonic genome. The high rate of glutamine metabolism at the early cleavage stages, followed by a significant decrease to a minimum at the blastocyst stage and then an increase with expansion, is consistent with the known importance of glutamine to the early development of hamster and mouse embryos. These observations support our previous suggestion (Rieger & Guay, 1988) that the role of glutamine as an energy substrate for cattle (and other mammalian) embryos deserves further study. Gardner *et al.* (1989) subsequently suggested that studies of the joint uptake of glucose and glutamine at the blastocyst stage are needed to guide further research on the role of glutamine in mammalian blastocysts. However, this view would seem to be too narrow; on the basis of our observations, the metabolism of glutamine and glucose may be more directly related to early embryonic development than is their uptake and, rather than being limited to the blastocyst stage,

the relationship between glucose and glutamine metabolism appears to be important from the earliest cleavage stages onward.

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