Utilization of [U-^{14}C] Glucose by Preimplantation Rat Embryo Cultured In Vitro

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Abstract: *The oxidation and incorportion of [U-^{14}C] glucose were examined in preimplantation rat embryos. An increasing capacity for incorporation and oxidation of glucose after the 1-cell stage was observed and the oxidative turnover of this substrate at the blastocyst stage was ten times more than that at the 1-cell stage. To evaluate how glucose is utilized for the synthesis of embryo lipids, 2-cell embryos and blastocysts were cultured for 5 h in medium containing [U-^{14}C] glucose, and total lipids extracted from the embryo were separated into various neutral lipids and phospholipids by thin layer chromatography and radioactivities of these lipid fractions were measured. Most of the radioactivity was recovered in triacylglycerols in both stages, and radioactivities were also found in other neutral lipids and phospholipids. These results indicate that [U-^{14}C] glucose was certainly utilized for oxidation and the synthesis of various lipids in embyros at the preimplamtation stage.*

Key words: *Incorporation of glucose, Distribution of glucose, Oxidation of glucose, Lipid synthesis, Rat embryo.*

The composition characteristics of several kinds of saturated and unsaturated fatty acids in rat embryo and oviduct and uterine fluids were reported [1]. Moreover, it was demonstrated that the development of rat embryos was seriously affected in the absence of fatty acids bound to BSA in the culture medium [2]. Furthermore, in the absence of carbohydrate substrates, either the free fatty acids or those added to the culture medium had stronger effects on the development of rat embryos than carbohydrate substrates added to the culture medium [3]. Flynn and Hillman [4] reported that lipid synthesis from [U-^{14}C] glucose could be performed in preimplantation mouse embryos cultured *in vitro*.

Received: September 2, 1998
Accepted: February 22, 1999
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The present study was undertaken to test the oxidation and to characterize the incorporation of [U-^{14}C] glucose by preimplantation rat embryos.

Materials and Methods

Animals and embryo collection

Eight week-old rats of Wistar Imamich strain were raised under controlled light conditions(12 h light:12 h darkness: light on at 06:00 A.M.). The females were mated naturally overnight with the males, and examined for the presence of vaginal plugs the next morning. One-, two-, four-, and eight cell embryos, morula and blastocysts were collected from mated females by the flushing of oviducts at 8, 32, 56 and 76 h and of the uterus at 84 and 108 h after ovulation, respectively.

Experiment 1

Incorporation and oxidation experiments with 18.5 KBq [U-^{14}C] glucose (Specific activity: 10 μM/mM) were performed according to the method of Brinster [5]. The embryos were pooled with medium and 10 embryos were transferred to 0.1 ml BMOC III containing radioactive glucose in one microtube, and 1 ml Hyamine in another microtube. The contents of the two microtubes were then transferred to a scintillation vial. The scintillation vials were airtight and incubated for 3 h at 37°C under 5% CO_2 in air. Then the reaction was stopped by the addition of cold PCA to a final concentration of 5%. The vials were left sealed at 4°C for 24 h. Ten ml scintillation cocktail was added to the scintillation vial and mixed well with hyamine. The acid insoluble materials were washed several times by millipore filtration (scwp 8 μ) with 5% PCA and ethyl alcohol. The incorporation and oxidation of ^{14}C-glucose were determinated in a scintillation counter and five to eight samples were used for each observation.

Experiment 2

Incorporation of ^{14}C-glucose into embryo lipids: Twenty 2-cell or blastocyst embryos were transferred to 100 μl drops of ^{14}C-glucose (23.1 KBq, specific activity: 10 μM/mM) medium which had been overlaid with silicone oil and pre-incubated for 0.5 h, and then cultured in an incubator (37°C, 5% CO_2, 95% air) for 5 h. The labeled embryos were washed 3 times with 500 μl non-radioactive BMOC III, pooled and stored at –20°C until measurement of radioactivity.

Determination of ^{14}C-glucose incorporated into the embryo lipids: All of the 100 embryos at the 2-cell or blastocyst stage were extracted for 1 h at room temperature with 14.5 ml of chloroform-methanol-water (2:2:1.8, by vol.) according to the procedure of Bligh & Dyer [6]. The aqueous layer was removed from the chloroform layer. The radioactivity of both the chloroform and aqueous layers was determined. The chloroform layer was evaporated to dryness in a vacuum, and then resolved in 100~250 μl of hexane. Aliquots of the extract were used for distributive analysis of fatty acid.

Distributive analysis of ^{14}C-glucose: The distribution of ^{14}C-glucose in neutral and polar lipids was carried out by thin-layer chromatography on 2.5 × 8.0 cm aluminum sheet silica gel 60 thin plates (Merck, Darmstadt, Germany) according to the method described by Khandoker [3]. For fractionation of the neutral lipid, the lipid extracts were developed in hexane-diethyl ether-glacial acetic acid (80:20:1, by vol.) at room temperature. Development of the polar lipid fraction was performed in methyl acetate-propan-1-ol-chloroform-methanol-0.25% aqueous KCl (25:25:25:10:9, by vol.). Appropriate lipid standards (Sigma Chemicals, USA) were added to the lipid extract as carriers to provide enough materials for detection on the thin-layer plates.

Table 1. Incorporation and oxidation of ^{14}C-glucose in early preimplantation rat embryos

Cell stage	Incorporation (CPM/embryo)	Oxidation (CPM/embryo)
1-Cell	464 ± 5.9[a]	29.2 ± 1.0[a]
2-cell	630 ± 6.4[b]	42.1 ± 0.9[b]
4-cell	720 ± 6.3[c]	48.3 ± 0.7[b]
8-cell	2,015 ± 9.8[d]	130.2 ± 0.8[c]
Morula	3,072 ± 11.1[e]	192.4 ± 1.9[d]
Blastocyst	5,400 ± 13.5[f]	306.9 ± 2.1[e]

Data are expressed as the mean ± SEM (n=4). Significant difference was found between the means having different letters in their superscripts in the same column (p<0.05).

Measurement of radioactivity

Each radioactive sample was placed in a scintillation vial, then 5 ml scintillation cocktail containing 0.5% 2, 5-diphenyloxazole and 0.03% 2, 2-p-phenylene-bis (5-phenyloxazole) in toluene was added to the vial. The radioactivity of all samples was determined in a liquid scintillation counter (LS-6500, Beckman Instruments, Inc., USA).

Statistical analysis

The data obtained were statistically analyzed by Student's *t*-test and expressed as the mean ± SEM.

Results

Incorporation and oxidation of ^{14}C-glucose in early preimplantation rat embryos are shown in Table 1. Incorporation and oxidation of ^{14}C-glucose were detected in assays with rat embryos at an early developmental stage. The rates of incorporation and oxidation were relatively constant from the 1-cell to the blastocyst stage (P<0.05). The rates clearly increased after the 8-cell stage.

Distribution of radioactivity from [U-^{14}C] glucose in the rat embryo lipids is shown in Table 2. The bulk (93.3%) of the label from ^{14}C-glucose was incorporated into the neutral lipid fraction by 2-cell stage embryos, but only 32.3% by blastocyst stage embryos. Major labeled neutral lipids was triacylglycerols at the 2-cell and blastocyst stages, 96.1 and 50.5% respectively

Table 2. Distribution of radioactivity from [U-^{14}C] glucose in rat embryo lipids

Fraction	Distribution of radioactivity (%)	
	Two-cell	Blastocyst
Neutral lipids		
Polar lipid at origin	0.5 ± 0.1	12.4 ± 2.3†
Monoacylglycerol	0.6 ± 0.1	8.7 ± 4.7
Fatty alcohol	0.9 ± 0.1	7.8 ± 3.5
Triacylglycerol	96.1 ± 5.4	50.5 ± 8.9†
Fatty acid	0.5 ± 0.5	3.8 ± 1.1†
Diacylglycerol	0.5 ± 0.7	12.1 ± 3.6†
Sterol ester	0.8 ± 0.3	4.7 ± 3.0
Phospholipids		
Lysophosphatidylcholine	24.7 ± 1.2	35.7 ± 19.4
Phosphatidylcholine	58.7 ± 9.8	14.1 ± 5.5
Phosphatidylinositol	12.8 ± 3.1	8.1 ± 4.5
Phosphatidylethanolamine	3.7 ± 0.9	42.1 ± 14.2

Data are expressed as the mean ± SEM. †Values differ significantly (p<0.05) from the corresponding ones at the two-cell stage.

($p<0.05$). Except for the labeled triacylglycerols, the amounts of neutral lipid classes labeled by ^{14}C-glucose were not significantly different from each other at the 2-cell stage. But the radioactivity was higher in polar lipids and several kinds of neutral lipids such as diacylglycerols, monoacylglycerols and fatty acids at the blastocyst stage than at the 2-cell stage ($p<0.05$). In the phospholipids, the fractions at the 2-cell and blastocyst stages were 6.1 and 67.7% of total radioactivity, respectively ($p<0.05$). Large amounts of radioactivity in the phospholipid fraction were recovered in phosphatidylcholines (58.7%) and lysophosphatidylcholines (24.7%) at the 2-cell stage, and in lysophosphatidylcholine (35.7%) at the blastocyst stage. These results indicate the [U-^{14}C] glucose was certainly utilized for oxidation and the synthesis of various lipids in embryos at the preimplantation stage.

Discussion

Rat embryo had a complete developmental blockage at the 2- or 4-cell stage *in vitro*. Tsujii and Takagi [7] and Kishi *et al.* [8] reported that the developmental block in cultured rat 1-cell embryos could be overcome by using a chemically defined medium, (hamster embryo culture medium1; HECM-1) [9]. We suggested that phosphate and glucose are responsible for the developmental block. Miyoshi *et al.* [10, 11] suggested that osmolarity is important for rat embryonic development, but it has been established that the concentration of glucose in uterine luminal fluid ranges from 0.02 to 1.5 mM in mouse [12], from 2.6 to 3.8 mM in sheep [13] and 5 mM in cow [14]. Although glucose does not support the development of early-cleavage-stage rat embryos, the beneficial effect of a low concentration of glucose on the development *in vitro* of cleavage-stage rat embryos has been documented [15]. Nevertheless, active utilization of glucose through the glycolysis and TCAcycle is reported to start a little before the blastocyst formation in rat embryo [16]. It seems therefore that glucose could be an important substrate for early preimplantation rat embryo, and the experiments presented here demonstrated the capacity of the 1- and 2-cell rat embryos to catalyze glucose to carbon dioxide.

The pattern of utilization of glucose from the 1-cell to the blastocyst stage in rat embryos indicates a substantial increase in the capacity to oxidize this substrate with development. The increasing capacity to oxidize glucose as development proceeds appears to be a general phenomenon with mammalian embryos and is also seen in mouse [5], rabbit [17], human [18], cow [19] and sheep [20] embryos. This increasing incorporation and CO_2 production of [U-^{14}C] glucose could arise from the increased pentose phosphate pathway (PPP) activity, increased TCA cycle processing, or a combination of both. The measurement of PPP activity in mouse embryo indicated that activity in this pathway increases with development [21].

Waugh and Wales [20] reported that the major incorporation of glucose carbon by cattle embryos occurs in the acid-soluble, non-glucose pool, and approximately 70% of total incorporation is found in this fraction with most of the remainder in non-glycogen macromolecules. The present data indicated that preimplantation rat embryos can incorporate the labels from [U-^{14}C] glucose into lipids. In close agreement with mouse embryos studies [4, 22, 23], glucose carbon in rat embryo lipogenesis is through glucose-3-phosphate into triacylglycerols.

Khandoker [3] reported that 8-cell rat embyos were cultured for 2 h in a medium containing ^{3}H-palmitic acid and total lipids extracted from the embryos were separated into various neutral and polar lipids by thin layer chromatography. More than 70% of radioactivity was recovered in triacylglycerols and 21% in fatty alcohol in neutral lipids, whereas more than 90% was recovered in choline phosphatides in polar lipids. Similar results were reported for mouse embryos [4, 24, 25].

A major biological role of triacylglycerols is to serve as a storage form of metabolic energy. Since preimplantation embryo readily ultilize other compounds (e.g. lactate, pyruvate, glucose) as energy sources during preimplantation development [26], it is possible that triacylglycerols are synthesized and stored by these embryos for a later period of development when they may not be capable of utilizing exogenous substrates. The triacylglycerols have a higher energy content per unit mass than glycogen. The accumulation of triacylglycerols during the early stages of rat embryogenesis may also have developmental significance since the accumulation of triacylglycerols also occurs during the early development of other vertebrates [26].

Similar synthesis of fatty acid and triacylglycerol and their elaborate regulatory control of somatic cells such as adipocytes may exist in mammalian embryos. In adipocytes the products of glycolysis are converted into fatty acids via the *de novo* biosynthetic pathway. Surplus citrate in the pathway is transported from the mitochondrion and cleaved to produce cytoplasmic acetyl-CoA is acted upon by acetyl-CoA carboxylase to produce malonyl-CoA. The next steps in the fatty acid biosynthetic pathway are carried out by the multifunctional fatty acid synthatase enzyme which utilizes NADPH to catalyze mutiple condensations of malonyl-CoA with

acetyl-CoA or the elongating lipid, eventually generating palmitate. Fatty acids are subsequently esterified with CoA and condensed with α-glycerol phosphate to generate triacylglycerol. This phosphorylation of perlipins by cAMP-dependent protein kinase [27] is correlated with the lipolytic process. The significant differences between triacylglycerol synthesis from glucose in the 2-cell and blastocyst stages suggest that active metabolism of triacylglycerol synthesis was carried out at the 2-cell stage for storing large amounts of energy substrates, and active triacylglycerol catabolism at the blastocyst stage was for supplying large amounts of metabolic energy for the development and differentiation of rat blastocyst.

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