

## **Incorporation and Distribution of Both $^3\text{H}$ -Palmitic and $^{14}\text{C}$ -Oleic Acids in Lipids by Preimplantation Mouse Embryos**

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**Abstract:** Both  $^3\text{H}$ -palmitic and  $^{14}\text{C}$ -oleic acids were simultaneously incorporated into mouse embryo lipids from the 1-cell to the blastocyst stage. Rates of incorporation of the two fatty acids into the embryo lipids increased and differed from each other significantly after 8-cell stage ( $P<0.05$ ). That of palmitic acid into the polar lipid fraction was significantly higher than that of oleic acid at all corresponding cell stages, but that of palmitic acid into the neutral lipids was significantly higher than that of oleic acid at the morula and blastocyst stages ( $P<0.05$ ). In the neutral lipid fraction, the radioactivity of  $^3\text{H}$ -palmitic acid distributed in triacylglycerols, monoacyldiglycerols and diacylglycerols were significantly higher than that of  $^{14}\text{C}$ -oleic acid at most corresponding cell stages with the exception of high monoacyldiglycerol content in  $^{14}\text{C}$ -oleic acid at the 2-cell stage ( $P<0.05$ ). But that of  $^3\text{H}$ -palmitic acid in fatty alcohols and monoacylglycerols was significantly lower than that of  $^{14}\text{C}$ -oleic acid at the most corresponding cell stages ( $P<0.05$ ). In the polar lipid fraction, the amounts of  $^3\text{H}$ -palmitic acid distributed in choline phosphatides, ethanolamine phosphatides and sphingomyelins were significantly greater at most corresponding cell stages than that of  $^{14}\text{C}$ -oleic acid ( $P<0.05$ ) but the radioactivity of  $^{14}\text{C}$ -oleic acid recovered in inositol or serine phosphatides, lysophosphatidylcholines, monoglycosylglycerides was significantly higher than that of  $^3\text{H}$ -palmitic acid at almost every corresponding cell stage ( $P<0.05$ ). These results demonstrated that the patterns of incorporation and distribution of double fatty acids in various lipid species were significantly different from those of the same single fatty acids in our previous report.

**Key words:** Palmitic acid, Oleic acid, Incorporation of fatty acids, Distribution of fatty acids, Mouse embryo.

It has been demonstrated that the addition of exogenous fatty acids (FAA) to culture medium has certain effects on the development of mouse [3, 6, 9, 10] and other mammalian embryos [5]. Flynn *et al.* [4] first indicated that exogenous palmitic acid could be incorporated into the embryo lipids of 8-cell staged mouse embryos to synthesize various lipid species. Khandoker *et al.* [7] reported that exogenous palmitic acid could be incorporated into rat embryo lipids and was recovered in various lipid classes in preimplantation rat embryos cultured *in vitro*. Wang *et al.* [16] revealed the metabolic differences among various cell stages of exogenous palmitic or oleic acid alone in preimplantation mouse embryos. Urade *et al.* [13] reported that lipid metabolism related to triacylglycerol and phospholipid species was perturbed by palmitic acid in Chinese Hamster V79-R cells in the presence of various fatty acids. The Mammalian embryo grows and develops in the simultaneous presence of various fatty acids under normal physiological conditions. The effects of inhibition and cooperation among various fatty acids in mammalian cells remains unknown. In particular no attempt has been made to reveal the metabolic characteristics of multi-fatty acids in preimplantation mouse embryos cultured *in vitro*.

In the present study, simultaneous incorporation of  $^3\text{H}$ -palmitic together with and  $^{14}\text{C}$ -oleic acids into lipids, and their distribution in mouse embryos at different cell stages, were characterized and compared with each other at the corresponding cell stages.

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## Materials and Methods

### *Animals and embryo collection*

Six- to eight-week-old male and female mice of Slc:ICR strain (SLC Co. Japan) were kept in controlled environmental conditions at room temperature with a cycle of 12 hs light (light on from 6:00 a.m. to 6:00 p.m.) and 12 hs dark. The females were superovulated by the routine method described elsewhere [15, 16]. The embryos of 1-cell, 2-cell, 8-cell, compact morula and early blastocyst embryos were collected from oviducts and uteri 24, 40, 65, 77 and 90 hs after hCG injection, respectively. The collected embryos were washed three times in M16 medium without BSA and were used for *in vitro* culture in labeled M16 medium.

### *Preparation of the labeled culture medium*

$^3\text{H}$ -palmitic and  $^{14}\text{C}$ -oleic acid-labeled medium was prepared by the method described by Flynn *et al.* [4]. 4.265 MBq of [9,10- $^3\text{H}$ ] palmitic acid (Specific activity: 1.85 TBq/mM; Moravsek Biochemicals, Inc.) and 4.265 MBq of [1- $^{14}\text{C}$ ] oleic acid (Specific activity: 2.04 TBq/mM; American Radiolabeled Chemicals Inc.) in ethyl alcohol were put into a sterile glass tube, and mixed well with 0.2 ml of 1 mM non-radioactive palmitic acid and 0.2 ml of oleic acids (Sigma Chemical Co. Ltd. St. Louis, USA) in benzene. The solvents in the tube were evaporated in a vacuum, then 4 ml of M16 medium containing 5 mg/ml fatty acid-free BSA (FAF-BSA, Sigma Chemical Co. Ltd. St. Louis, USA) was added. The tube was then placed in a Ultra-Sonic Cleaner (USC-1, Japan) bath and sonicated for 0.5 h. Aliquots of the labeled medium was filtered with a 0.22  $\mu\text{m}$  filter and stored at 4°C before use. The concentration of total fatty acids in this medium was 0.1 mM, and the molar ratio of fatty acids to albumin was about 1:2.

### *Incorporation of exogenous fatty acids into embryo lipids*

Twenty each 1-cell, 2-cell, 8-cell, morula and blastocyst embryos were transferred in a 100  $\mu\text{l}$  drop of the  $^3\text{H}$ -palmitic and  $^{14}\text{C}$ -oleic acid-labeled medium which had been overlaid with silicone oil and pre-incubated for 1 h, and then cultured in an incubator (37°C, 5%  $\text{CO}_2$ , 95% air) for 3 h. The labeled embryos were washed 5 times by pipetting in non-radioactive M16 medium without BSA, pooled and stored at -20°C until measurement of radioactivity.

### *Radioactive detection of the labeled embryo lipids*

About 200 labeled embryos at each individual cell stage were extracted according to the procedure of Bligh *et al.* [2]. The aqueous layer was removed from the chloroform layer. The total radio activity of the chloroform layer was detected, and then it was evaporated to dryness in a vacuum. The lipid extract was taken up in 100~250  $\mu\text{l}$  of hexane. Each extract was used for its distributive analysis by thin-layer chromatography.

### *Distributive detection of $^3\text{H}$ -palmitic and $^{14}\text{C}$ -oleic acids*

Distribution of  $^3\text{H}$ -palmitic and  $^{14}\text{C}$ -oleic acids was analyzed by thin-layer chromatography on 60 thin 2.5  $\times$  8.0 cm aluminum sheet silica gel plates (Merck, Darmstadt, Germany) by the method described by Wang *et al.* [16]. For the fractionation of neutral lipids, 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 class was performed in methylacetate-propan-1-ol-chloroform-methanol-0.25% KCl (25:25:25:10:9, by vol.). Appropriate lipid standards (Sigma Chemicals, USA) were added to the lipid extract as carriers for the location of individual lipid species.

### *Measurement of radioactivity*

Radioactivity of all samples were determined in a liquid scintillation counter (LS-6500, Beckman Instruments, Inc., USA) by the routine method described elsewhere [7, 16].

### *Statistical analysis*

The data were statistically analyzed by Fisher's test and expressed as mean  $\pm$  SEM.

## Results

Rates of incorporation of both  $^3\text{H}$ -palmitic and  $^{14}\text{C}$ -oleic acids into embryo lipids at various cell stages are shown in Table 1. The results showed that two fatty acids were constantly incorporated into the embryo lipids from the 1-cell to the blastocyst stage, and increased significantly from the 8-cell to the blastocyst stage ( $P < 0.05$ ). The rates of incorporation of  $^3\text{H}$ -palmitic acid into the embryo lipids were significantly higher than those of  $^{14}\text{C}$ -oleic acid after the 8-cell stage ( $P < 0.05$ ), and that of  $^3\text{H}$ -palmitic acid into the neutral lipid fraction was significantly higher than that of  $^{14}\text{C}$ -oleic acid after the morula stage ( $P < 0.05$ ). But that of  $^3\text{H}$ -palmitic acid into the polar lipid fraction was significantly higher than that of  $^{14}\text{C}$ -oleic acid at all corresponding cell stages ( $P < 0.05$ ).

The distribution of  $^3\text{H}$ -palmitic and  $^{14}\text{C}$ -oleic acids in

**Table 1.** The rates of incorporation of both  $^3\text{H}$ -palmitic and  $^{14}\text{C}$ -oleic acids into embryo lipids at individual cell stages

Cell stage	fmol/embryo/h					
	In total lipid		In neutral lipid		In polar lipid	
	Palmitic	Oleic	Palmitic	Oleic	Palmitic	Oleic
One-cell	58.5 $\pm$ 4.1 <sup>a</sup>	61.1 $\pm$ 5.7 <sup>a</sup>	42.2 $\pm$ 3.4 <sup>a</sup>	49.2 $\pm$ 2.1 <sup>ab</sup>	16.3 $\pm$ 3.2 <sup>a</sup>	12.0 $\pm$ 2.6 <sup>b</sup>
Two-cell	61.1 $\pm$ 5.2 <sup>a</sup>	57.7 $\pm$ 4.3 <sup>a</sup>	41.1 $\pm$ 4.3 <sup>a</sup>	44.9 $\pm$ 3.9 <sup>a</sup>	20.0 $\pm$ 1.5 <sup>a</sup>	12.8 $\pm$ 2.0 <sup>b</sup>
Eight-cell	78.0 $\pm$ 3.0 <sup>a</sup>	63.7 $\pm$ 3.2 <sup>b</sup>	53.1 $\pm$ 3.7 <sup>a</sup>	53.1 $\pm$ 4.3 <sup>a</sup>	24.9 $\pm$ 2.8 <sup>a</sup>	10.6 $\pm$ 2.8 <sup>b</sup>
Morula	113.1 $\pm$ 3.6 <sup>a</sup>	97.5 $\pm$ 4.6 <sup>b</sup>	83.1 $\pm$ 2.9 <sup>a</sup>	75.0 $\pm$ 2.3 <sup>b</sup>	30.0 $\pm$ 2.7 <sup>a</sup>	19.5 $\pm$ 2.8 <sup>b</sup>
Blastocyst	223.6 $\pm$ 8.7 <sup>a</sup>	170.3 $\pm$ 6.7 <sup>b</sup>	168.4 $\pm$ 3.2 <sup>a</sup>	138.3 $\pm$ 3.6 <sup>b</sup>	55.2 $\pm$ 2.3 <sup>a</sup>	32.0 $\pm$ 3.0 <sup>b</sup>

a, b: Superscript values within the same row of each column differ significantly from each other ( $P < 0.05$ ). The data are expressed as the mean  $\pm$  SEM. The results were obtained from four determinations in two independent experiments utilizing about 200 embryos in vitro culture medium.

**Table 2.** Distribution of  $^3\text{H}$ -palmitic and  $^{14}\text{C}$ -oleic acids in neutral lipids at individual cell stages

Fraction	total neutral lipids at several cell stages									
	One-cell		Two-cell		Eight-cell		Morula		Blastocyst	
	$^3\text{H}$ -PA	$^{14}\text{C}$ -OA	$^3\text{H}$ -PA	$^{14}\text{C}$ -OA	$^3\text{H}$ -PA	$^{14}\text{C}$ -OA	$^3\text{H}$ -PA	$^{14}\text{C}$ -OA	$^3\text{H}$ -PA	$^{14}\text{C}$ -OA
TG	32.2 $\pm$ 3.0 <sup>a</sup>	22.0 $\pm$ 2.2 <sup>b</sup>	34.5 $\pm$ 1.9 <sup>a</sup>	27.2 $\pm$ 2.9 <sup>bc</sup>	28.4 $\pm$ 3.1 <sup>a</sup>	18.5 $\pm$ 2.3 <sup>bd</sup>	32.9 $\pm$ 3.6 <sup>a</sup>	19.2 $\pm$ 1.8 <sup>bd</sup>	34.7 $\pm$ 3.9 <sup>a</sup>	24.8 $\pm$ 2.0 <sup>b</sup>
MG	13.7 $\pm$ 2.3 <sup>a</sup>	14.1 $\pm$ 2.0 <sup>a</sup>	15.8 $\pm$ 3.2 <sup>a</sup>	20.8 $\pm$ 3.0 <sup>b</sup>	12.9 $\pm$ 2.8 <sup>a</sup>	11.0 $\pm$ 2.6 <sup>a</sup>	20.6 $\pm$ 3.4 <sup>b</sup>	31.7 $\pm$ 3.6 <sup>c</sup>	16.6 $\pm$ 1.2 <sup>a</sup>	20.0 $\pm$ 2.0 <sup>b</sup>
MADG	12.9 $\pm$ 2.3 <sup>a</sup>	8.4 $\pm$ 1.9 <sup>b</sup>	8.5 $\pm$ 2.1 <sup>b</sup>	15.5 $\pm$ 3.0 <sup>a</sup>	10.5 $\pm$ 2.3 <sup>a</sup>	6.7 $\pm$ 1.1 <sup>bc</sup>	9.0 $\pm$ 1.8 <sup>b</sup>	6.3 $\pm$ 2.7 <sup>bc</sup>	11.4 $\pm$ 2.9 <sup>a</sup>	5.3 $\pm$ 1.0 <sup>bc</sup>
DG	2.6 $\pm$ 0.6 <sup>a</sup>	0.7 $\pm$ 0.3 <sup>b</sup>	8.6 $\pm$ 1.9 <sup>c</sup>	0.2 $\pm$ 0.1 <sup>d</sup>	2.2 $\pm$ 0.3 <sup>a</sup>	1.9 $\pm$ 0.4 <sup>1a</sup>	2.8 $\pm$ 0.8 <sup>a</sup>	0.1 $\pm$ 0.1 <sup>d</sup>	3.8 $\pm$ 0.9 <sup>a</sup>	2.8 $\pm$ 0.6 <sup>ae</sup>
FCO	27.02 $\pm$ 3.9 <sup>a</sup>	30.2 $\pm$ 2.7 <sup>a</sup>	19.6 $\pm$ 2.1 <sup>b</sup>	25.3 $\pm$ 3.2 <sup>a</sup>	23.2 $\pm$ 2.9 <sup>a</sup>	26.8 $\pm$ 3.7 <sup>a</sup>	27.9 $\pm$ 3.4 <sup>a</sup>	38.8 $\pm$ 4.5 <sup>c</sup>	27.5 $\pm$ 2.0 <sup>a</sup>	38.5 $\pm$ 3.3 <sup>c</sup>
FAA	13.2 $\pm$ 1.9 <sup>a</sup>	9.3 $\pm$ 2.9 <sup>b</sup>	7.9 $\pm$ 1.8 <sup>b</sup>	7.9 $\pm$ 2.0 <sup>b</sup>	8.3 $\pm$ 2.4 <sup>b</sup>	10.4 $\pm$ 1.9 <sup>ab</sup>	4.9 $\pm$ 1.1 <sup>c</sup>	3.9 $\pm$ 0.8 <sup>c</sup>	6.0 $\pm$ 1.7 <sup>b</sup>	7.1 $\pm$ 1.5 <sup>b</sup>
SE	UD <sup>a</sup>	0.3 $\pm$ 0.2 <sup>b</sup>	5.1 $\pm$ 1.8 <sup>c</sup>	3.4 $\pm$ 1.1 <sup>cd</sup>	14.6 $\pm$ 2.4 <sup>e</sup>	24.7 $\pm$ 3.6 <sup>f</sup>	1.9 $\pm$ 0.7 <sup>dg</sup>	UD <sup>a</sup>	UD <sup>a</sup>	1.5 $\pm$ 0.8 <sup>dg</sup>

a~g: Superscript values within the same row differ significantly from each other ( $P < 0.05$ ).  $^3\text{H}$ -PA and  $^{14}\text{C}$ -OA mean  $^3\text{H}$ -Palmitic and  $^{14}\text{C}$ -oleic acids, respectively. TG: Triacylglycerols; MG: Monoacylglycerols; MADG: Monoacyldiacylglycerols; DG: Diacylglycerols; FCO: Fatty alcohols; FAA: Fatty acids; SE: Sterol esters. All values were obtained from four determinations of three independent experiments utilizing 200 embryos in each.

the neutral lipid fraction is shown in Table 2. Large amounts of  $^3\text{H}$ -palmitic and  $^{14}\text{C}$ -oleic acids were mainly recovered in triacylglycerols (TGs), followed by fatty alcohols (FCOs), monoacylglycerols (MGs) and monoacyldiacylglycerols (MADGs), and small amounts were found in the other neutral lipid classes. The radioactivity of TGs and DGs classes from  $^3\text{H}$ -palmitic acid was significantly higher than that of TGs and DGs from  $^{14}\text{C}$ -oleic acid at all corresponding cell stages ( $P < 0.05$ ), and that of  $^3\text{H}$ -palmitic acid in MADGs were also significantly higher than that of  $^{14}\text{C}$ -oleic acid at most corresponding cell stages ( $P < 0.05$ ) with the exception of lower radioactivity of  $^3\text{H}$ -palmitic acid than that of  $^{14}\text{C}$ -oleic acid at the 2-cell stage but the radioactivity of  $^{14}\text{C}$ -oleic acid in fatty alcohol and MGs was significantly higher than that of  $^3\text{H}$ -palmitic acid at most corresponding cell stages ( $P < 0.05$ ). The amounts of free  $^3\text{H}$ -palmitic and  $^{14}\text{C}$ -oleic acids incorporated into the embryos were maintained constantly and not significantly different from

each other at the same cell stages ( $P < 0.05$ ). Very small amounts of sterol esters (SEs) from both  $^3\text{H}$ -palmitic and  $^{14}\text{C}$ -oleic acid appeared at the 1-cell, morula and blastocyst stages. But very large amounts of the labeled SEs were observed at the 8-cell stage, and the radioactivity of  $^{14}\text{C}$ -oleic in SEs was significantly higher than that of  $^3\text{H}$ -palmitic acid at this stage ( $P < 0.05$ ).

The distributions of  $^3\text{H}$ -palmitic and  $^{14}\text{C}$ -oleic acids in the polar lipid fraction are shown in Table 3. Both  $^3\text{H}$ -palmitic and  $^{14}\text{C}$ -oleic acids were mainly recovered in choline phosphatides (PCs), sphingomyelins (SGMs), inositol phosphatides (PIs)-serine phosphatides (PSSs), ethanamines phosphatides (PEs) and monoglycosylglycerides (MGGs). But the radioactivity of  $^3\text{H}$ -palmitic acid in PCs, SGMs and PEs was significantly higher than that of  $^{14}\text{C}$ -oleic acid at most corresponding cell stages ( $P < 0.05$ ), whereas that of  $^{14}\text{C}$ -oleic acid in PIs-PSSs, MGGs and lysophosphatidylcholines (LPCs) species was significantly higher than that of  $^3\text{H}$ -palmitic

**Table 3.** Distribution of  $^3\text{H}$ -palmitic and  $^{14}\text{C}$ -oleic acids in polar lipids at individual cell stages

Fraction	total polar lipids at several cell stages (%)									
	One-cell		Two-cell		Eight-cell		Morula		Blastocyst	
	$^3\text{H}$ -PA	$^{14}\text{C}$ -OA	$^3\text{H}$ -PA	$^{14}\text{C}$ -OA	$^3\text{H}$ -PA	$^{14}\text{C}$ -OA	$^3\text{H}$ -PA	$^{14}\text{C}$ -OA	$^3\text{H}$ -PA	$^{14}\text{C}$ -OA
PPL										
PC	19.5 ± 2.3 <sup>a</sup>	17.7 ± 2.6 <sup>a</sup>	37.7 ± 6.8 <sup>b</sup>	18.6 ± 2.7 <sup>a</sup>	16.0 ± 3.1 <sup>a</sup>	8.8 ± 2.9 <sup>c</sup>	28.9 ± 2.5 <sup>d</sup>	21.3 ± 3.1 <sup>e</sup>	29.1 ± 2.5 <sup>d</sup>	25.0 ± 1.6 <sup>de</sup>
SGM	13.5 ± 1.4 <sup>a</sup>	10.4 ± 1.0 <sup>b</sup>	24.1 ± 3.3 <sup>c</sup>	19.6 ± 2.8 <sup>d</sup>	19.6 ± 2.5 <sup>d</sup>	10.3 ± 1.8 <sup>b</sup>	25.2 ± 3.7 <sup>c</sup>	9.2 ± 2.6 <sup>b</sup>	10.8 ± 2.1 <sup>b</sup>	8.0 ± 2.8 <sup>b</sup>
PI/PS	9.8 ± 1.2 <sup>a</sup>	17.6 ± 2.5 <sup>b</sup>	14.3 ± 2.0 <sup>bc</sup>	18.3 ± 1.6 <sup>b</sup>	27.2 ± 1.5 <sup>d</sup>	34.1 ± 1.8 <sup>c</sup>	6.6 ± 1.7 <sup>af</sup>	12.1 ± 2.0 <sup>bc</sup>	9.1 ± 2.1 <sup>a</sup>	17.7 ± 1.7 <sup>b</sup>
PE	11.9 ± 2.3 <sup>a</sup>	4.8 ± 1.0 <sup>b</sup>	8.7 ± 1.2 <sup>ac</sup>	6.9 ± 1.7 <sup>ab</sup>	13.1 ± 2.0 <sup>ad</sup>	9.4 ± 2.3 <sup>ac</sup>	15.3 ± 2.1 <sup>ad</sup>	11.3 ± 1.9 <sup>a</sup>	5.6 ± 1.1 <sup>b</sup>	3.3 ± 0.5 <sup>be</sup>
LPC	1.6 ± 1.2 <sup>a</sup>	3.4 ± 0.8 <sup>b</sup>	4.6 ± 1.3 <sup>b</sup>	8.8 ± 1.9 <sup>c</sup>	6.3 ± 1.7 <sup>c</sup>	10.3 ± 2.0 <sup>d</sup>	1.9 ± 0.8 <sup>a</sup>	7.8 ± 1.9 <sup>c</sup>	13.4 ± 2.5 <sup>de</sup>	13.3 ± 2.4 <sup>de</sup>
GCL										
MGG	15.2 ± 2.5 <sup>a</sup>	16.5 ± 2.7 <sup>a</sup>	8.8 ± 2.1 <sup>b</sup>	13.6 ± 1.5 <sup>a</sup>	4.7 ± 1.5 <sup>c</sup>	16.8 ± 3.2 <sup>a</sup>	9.7 ± 2.0 <sup>b</sup>	25.5 ± 3.9 <sup>d</sup>	16.3 ± 2.0 <sup>a</sup>	13.3 ± 1.4 <sup>ac</sup>
SFT	9.9 ± 2.1 <sup>a</sup>	5.4 ± 1.2 <sup>b</sup>	1.8 ± 0.7 <sup>c</sup>	12.7 ± 2.6 <sup>d</sup>	10.3 ± 1.9 <sup>d</sup>	UD <sup>e</sup>	4.4 ± 1.2 <sup>b</sup>	7.8 ± 1.6 <sup>a</sup>	7.2 ± 2.1 <sup>a</sup>	8.7 ± 2.2 <sup>a</sup>
CBS	18.6 ± 1.7 <sup>a</sup>	24.2 ± 2.1 <sup>b</sup>	UD <sup>c</sup>	1.0 ± 0.6 <sup>d</sup>	3.3 ± 1.2 <sup>e</sup>	10.3 ± 2.8 <sup>f</sup>	7.9 ± 2.1 <sup>fg</sup>	5.0 ± 1.3 <sup>e</sup>	8.5 ± 1.9 <sup>fg</sup>	9.9 ± 2.0 <sup>fg</sup>

a~g: Superscript values within the same row differ significantly from each other.  $^3\text{H}$ -PA and  $^{14}\text{C}$ -OA mean  $^3\text{H}$ -palmitic and  $^{14}\text{C}$ -oleic acids, respectively. UD: undetectable. PPL: Phospholipid; PC: choline phosphatides; SGM: Spingomyelin; PI/PS: The mixtures of phosphatidylinositols and phosphatidylserines which could not be fractionated from each other very well on the thin-layer chromatography plates; PE: Ethanolamines; LPC: lysophosphatidylcholines; GCL: Glycolipids; MGG: Monoglycosylglycerides; SFT: Sulfatides; CBS: Cerebrosides. All values were obtained from four determinations in three independent experiments utilizing about 200 labeled embryos in each.

acid ( $P < 0.05$ ). Distributions of  $^3\text{H}$ -palmitic and  $^{14}\text{C}$ -oleic acids in sulfatides and cerebrosides were quite different from each other and varied significantly according to the cell stage ( $P < 0.05$ ).

### Discussion

The present study revealed that preimplantation mouse embryos at different cell stages had the ability to simultaneously utilize exogenous palmitic and oleic acids by incorporating them into the embryo lipid fractions and distributing them into various lipid classes. Nevertheless, the incorporate rates of both  $^3\text{H}$ -palmitic and  $^{14}\text{C}$ -oleic acid into the embryo lipids and their patterns of distribution in the neutral and polar lipid fractions differed significantly from those of the single administration of palmitic or oleic acid and the patterns at the same cell stages.

The rates of incorporation of palmitic acid into the embryo lipids, including the neutral and the polar lipid fractions, were significantly higher than those of oleic acid at some corresponding cell stages in both single and double culture medium. In the presence of palmitic and oleic acids in culture medium, the rates of incorporation of palmitic acid into the embryo lipids were significantly higher than that of oleic acid after the 8-cell stage, whereas that of palmitic acid into the embryo lipids was found to be significantly higher than that of oleic acid from the 1-cell to the morula stage with the exception of the blastocyst stage in the presence of

palmitic acid or oleic acid alone [16]. The amounts of both palmitic and oleic acids distributed in the neutral fraction increased significantly from only the 8-cell stage to the blastocyst stage, whereas that of either palmitic acid or oleic acid alone increased significantly from the 1-cell to the blastocyst stage [16]. In addition, the rates of incorporation of either palmitic acid or oleic acid into the embryo lipids were also influenced by the concentration in the culture medium. In the presence of 0.05 mM of both palmitic and oleic acids in the same culture, the incorporate rates of both palmitic and oleic acids were significantly lower than that of 0.1 mM of palmitic or 0.1 mM oleic acid alone in the culture at all corresponding cell stages [16]. Spector [11, 12] proposed that the incorporation of individual fatty acids into mammalian cells depended on the concentration and the chain saturation. In other words, a high fatty acid/albumin molar ratio and chain saturation are beneficial to the incorporation of fatty acid into the embryo lipids. Urade *et al.* [13] also observed that palmitic acid could be predominately incorporated into the cellular lipid in the culture of Chinese Hamster V79-R cells in the presence of oleic and palmitic acids.

The patterns of distribution of palmitic and oleic acids in individual neutral lipid species also showed some significant differences between the two fatty acids and single and double cultures. Our results for the double culture demonstrated that about 1/3 TG class were labeled by  $^3\text{H}$ -palmitic, and less TG class was labeled by  $^{14}\text{C}$ -oleic acid at all cell stages. Besides this, large

amounts of  $^3\text{H}$ -palmitic and  $^{14}\text{C}$ -oleic acids existed in FCO, MG and MADG classes. But in the single culture medium, more than 2/3 TG classes were labeled by  $^3\text{H}$ -palmitic acid, a little radioactivity of  $^3\text{H}$ -palmitic acid was recovered in the other neutral lipid classes. The radioactivity of  $^{14}\text{C}$ -oleic acid in the TG class in double-labeled culture was significantly lower than in the single-labeled culture [16], whereas the FCO class labeled by  $^{14}\text{C}$ -oleic acid was significantly higher than that in the single-labeled culture. In addition, the patterns of distribution of double  $^3\text{H}$ -palmitic and  $^{14}\text{C}$ -oleic acid in DG, MG, MADG and SE classes were not in accordance with that in the single-labeled culture at most corresponding cell stages [16]. These significant differences between single and double fatty acids in distribution were also found in other mammalian cells [12, 13]. The results of the present study were also supported by Urade *et al.*'s observation [13] that about 70% of TG species in Chinese Hamster V79-R cells were those containing three saturated palmitic acids or two saturated and one unsaturated oleic acid when palmitic and oleic acids were simultaneously present in the culture medium.

The patterns of distribution of  $^3\text{H}$ -palmitic and  $^{14}\text{C}$ -oleic acids in various polar lipid species also showed some significant differences between two fatty acids and single and double cultures. It is known that mammalian embryos have the different abilities to synthesize phospholipids [1, 14]. The results of the double-labeled culture demonstrated that  $^3\text{H}$ -palmitic acid and  $^{14}\text{C}$ -oleic acids were all widely distributed in various polar lipid species, but radioactivities of  $^3\text{H}$ -palmitic acid recovered in PC, SGM and PE classes were significantly higher than those of  $^{14}\text{C}$ -oleic acid at corresponding cell stages, whereas those of  $^3\text{H}$ -palmitic acid in PI/PS and LPC classes were significantly lower than those of  $^{14}\text{C}$ -oleic acid at most cell stages. Nevertheless, in the presence of a single fatty acid, large amounts of  $^3\text{H}$ -palmitic acid (57.0 to 71.5%) were mainly distributed in PC and PE classes, and small amounts (29.5 to 43.0%) of  $^3\text{H}$ -palmitic acid were recovered in the other phospholipids and glycolipids at all cell stages. Urade *et al.* [13] also indicated that the supplementation of palmitic acid caused an increase in the proportion of palmitic acid with a concomitant decrease in oleic acid in PC and PE classes. It still remains unknown what physiological significance oleic acid distributed largely in the mixtures of PI and PS classes has. But Mami *et al.* [8] reported that the activity of DNA polymerase  $\epsilon$  was selectively inhibited by PI and PS, and the inhibiting effect was obviously regulated by the fatty acid composition of PI

and PS classes, and the abundance of unsaturated fatty acids in PI and PS led to some promotional effects on the conversion of ADP into ATP and DNA duplication in mammalian cells.

Our study is the first to reveal the differences in simultaneous incorporation and distribution of palmitic and oleic acids in embryo lipids. The result suggest that the differences in incorporation and distribution in various embryo lipid species may be caused by selective uptake by embryos of fatty acid classes, fatty acid concentrations and the inhibition or cooperation of palmitic and oleic acid. Different fatty acid compositions in the culture medium in particular could influence the patterns of incorporation and distribution of exogenous fatty acids in the embryo lipids of preimplantation mouse embryo, and this may play an important role in regulating some characteristics of energy metabolism and the further development of embryos.

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