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Incorporation of ^{14}C–L–Leucine in Rat Eggs of Preimplantation Stage

III. Effects of Addition of Lactate and Succinate

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Summary

The effect of lactate and succinate on the incorporation of ^{14}C-L-Leucine in vitro was examined in rat eggs at three stages.

The addition of lactate has increased the incorporation of ^{14}C-L-Leucine in 1-cell, 2-cell and early blastocyst stages, respectively.

The addition of succinate has no effect on the incorporation of ^{14}C-L-Leucine in 1-cell and 2-cell stages, but increased the incorporation in the early blastocyst stage.

The addition of lactate has indicated a higher incorporation of Leucine than that in which succinate was added as an energy source.

We have previously studied the incorporation of ^{14}C-L-Leucine in rat eggs of the preimplantation stage (1, 2). In those results, the addition of glucose as an energy source had no effect on the incorporation of ^{14}C-L-Leucine in 1-cell and 2-cell eggs, but increased the incorporation in early blastocysts. Then, it seems that the effects of additional energy source substance on the incorporation of ^{14}C-L-Leucine is different in each stage of the eggs.

It has been observed that lactate and pyruvate are necessary substrates for the development of mouse egg in vitro (3–6). The present investigations were undertaken to examine the effects on the incorporation of ^{14}C-L-Leucine in the early embryonic stages of rat eggs of an addition of lactate and succinate to the medium as energy source.

Materials and Methods

All animals used here were females rats of the Wistar strain. The eggs used in this experiment were 1-cell (unfertilized), 2-cell and early blastocyst. 1- cell eggs were obtained from superovulated immature female rats at 28 to 29 hrs. after HCG injection. 2-cell eggs were obtained from superovulated virginal female rats, on day 2 of pregnancy, approximately 48 hrs. after HCG injection. Early blastocysts were

obtained from adult female rats on day 5 of pregnancy, approximately 102 hrs. after the estimated times of fertilization. The basic medium used in the study was Ca-free Krebs Ringer Phosphate Buffer (pH 7.4). The same solution was used for all manipulations. The handling of the embryos has been described previously (1, 2). The eggs collected in a watchglass were examined under a microscope and the normal eggs were transfered into 0.2 ml of solution in a centrifuge tube with a capillary pipett. The number of eggs used for measurement were 50 per sample (1, 2). 1 to 1.5 hours was taken for the manipulation. After preincubation at 37.5°C for 10 minutes, 0.1 ml lactate or succinate and 0.5 μci/ml universal labelled ^{14}C-L-Leucine (spec. act. 0.396 mci/ml) were added at the same time. These samples were incubated for 0, 0.5 and 1 hrs. The final concentration of sodium lactate was 5×10^{-2} or 5×10^{-3}M and of sodium succinate was 10^{-1} M, which are generally used for mammalian eggs (7, 8).

After each incubation time, the reaction was stopped by addition of cold TCA to a final concentration of 5 percent. Then following Millipore filtration (scwp 8μ), the acid insoluble material was washed with 5 percent TCA. The filtrate was dried under an infra red lamp and transfered to the vials for determination of radioactivity by a liquid scintillation counter (Packard Tri-Carb), as described previously (1, 2). The real incorporation value was estimated to be the measured value minus the 0- time value. The viability of the eggs after incubation was estimated by its staining affinity in nigrosin (0.03%) (9).

Results

Effects of lactate addition on the incorporation of ^{14}C-L-Leucine: – The optimum concentration of lactate for isotope incorporation was determined 1-cell eggs. The results are shown in Table 1. The effects of lactate addition on the incorporation of ^{14}C-L-Leucine were better in 5×10^{-2}M than in 5×10^{-3}M. Then, the effects of 5×10^{-2}M lactate addition on the incorporation of ^{14}C-L-Leucine were examined in 1-cell, 2-cell and early blastocyst stages. The results are shown in Table 2. The values of 1-cell and 2-cell egg and early blastocyst at 0.5 and 1 hr. of incubation were significant statistically ($p<0.05$) when compared with that of

TABLE 1. *Effect of Lactate* (5×10^{-3} or 5×10^{-2} *M*) *Addition on Incorporation of ^{14}C-L-Leucine in 1-cell*

	CPM/egg (M.±S.E.)	
Incubation time (hr.) / Conc. of Lactate	0.5	1
5×10^{-3} M	59.1±4.2 (8)	63.6±3.9 (8)
5×10^{-2} M	80.0±2.3 (8)	88.8±3.7 (8)

( ): No. of observation

TABLE 2. *Effect of Lactate (5×10^{-2} M) Addition on Incorporation of ^{14}C-L-Leucine*

	CPM/egg (M.±S.E.)	
Incubation time (hr.) / Stage of egg	0.5	1
1 - cell (unfertilized)	80.0±2.3(8)	88.8±3.7(8)
2 - cell	88.9±3.5(7)	103.5±8.4(7)
Early Blastocyst	128.2±2.1(8)	160.6±9.2(8)

( ): No. of observation

TABLE 3. *Effect of Succinate (10^{-1} M) Addition on Incorporation of ^{14}C-L-Leucine*

	CPM/egg (M.±S.E.)	
Incubation time (hr.) / Stage of egg	0.5	1
1 - cell (unfertilized)	46.5±3.1(5)	64.3±12.4(4)
2 - cell	49.4±2.3(6)	68.5± 9.2(5)
Early Blastocyst	96.6±5.7(6)	106.4± 7.1(6)

( ): No. of observation

^{14}C-L-Leucine alone (2). It has also been observed that the incorporation of ^{14}C-L-Leucine in 1-cell, 2-cell and early blastocysts is affected by the addition of 5×10^{-2}M lactate.

Effects of succinate addition on the incorporation of ^{14}C-L-Leucine: – The results obtained here are shown in Table 3. The significant increases of incorporation were observed in 1-cell, 2-cell and early blastocysts at 1 hr. of incubation as compared with 0.5 hr. ($P<0.05$). The incorporation in 1-cell and 2-cell stages in the case of succinate addition was not statistically different when compared with only ^{14}C-L-Leucine at 0.5 and 1 hrs. incubation, respectively ($P>0.05$). On the other hand, early blastocysts showed a significant increase of incorporation of ^{14}C-L-Leucine when 10^{-1} M succinate was added to the incubation medium when compared with only ^{14}C-L-Leucine at 1 hr. of incubation ($P<0.05$).

Discussion

Fig. 1 shows the effects of the addition of glucose (2), lactate and succinate on the incorporation of ^{14}C-L-Leucine compared with only ^{14}C-L-Leucine (2), respectively.

The addition of lactate to 1-cell, 2-cell and early blastocysts affected the

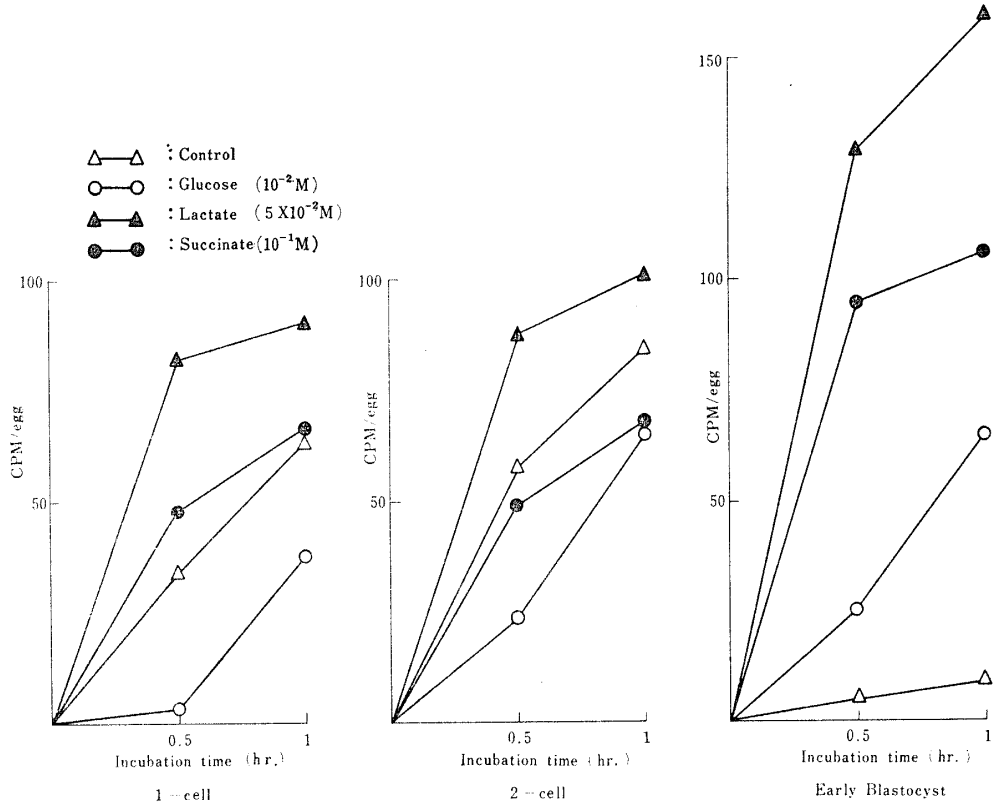

FIG. 1. The effects of the addition of glucose, lactate and succinate on the incorporation of ^{14}C-L-Leucine, as compared with only ^{14}C-L-Leucine.

incorporation of ^{14}C-L-Leucine. Especially, early blastocysts indicate a high incorporation of ^{14}C-L-Leucine at 0.5 and 1 hr. incubation. The rate of incorporation of ^{14}C-L-Leucine in the case of addition of lactate at 0.5 hr. of incubation was the same as that with only ^{14}C-L-Leucine at 3 hr. of incubation (2). Thus, an acceleration of the incorporation of ^{14}C-L-Leucine has observed with the addition of lactate. These increases of incorporation are coincident with the increase of resporatory quotient in the early blastocysts (10). It has been shown that 2-cell eggs of mouse develop in a medium containing only lactate as an energy source, but do not develop in a medium containing only glucose (3–6). And the production of cabon dioxide from lactate markedly increased up to the time of implantation in the mouse egg (7, 11). It may be suggested that lactate is an important energy source for rat as for preimplanted mouse egg.

When 10^{-1} M succinate was added to the incubation medium, the incorporation of ^{14}C-L-Leucine in 1-cell and 2-cell eggs was not affected, but early blastocysts were affected. Sugawara (8) has observed that the resporatory quotient in egg of rat was not affected with the addition of succinate until morula stage, but was affected in blastocyst stage. This report corresponds with the results indicating that the utilization of succinate has different affects on the early cleavage stage and on the blastocysts. The incorporation of ^{14}C-L-Leucine in 1-cell and 2-cell

stages was affected by the addition of 5×10^{-2} M lactate, but not affected by the addition of 10^{-1} M succinate and 10^{-2} M glucose when compared with only ^{14}C-L-Leucine, as shown in Fig. 1. On the other hand, early blastocysts were markedly affected by the addition of 5×10^{-2} lactate, 10^{-1} M succinate and 10^{-2} M glucose compared with that of only ^{14}C-L-Leucine. Thus, it may be suggested that the basic metabolism of egg in the early cleavage stage and blastocysts is different. We have investigated free amino acids in the uterine fluid of rat (12), and observed that the leucine in the uterine fluid increases before and after the implantation stage. This increase of leucine in the uterine fluid may be related to the higher incorporation of ^{14}C-L-Leucine in early blastocysts.

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