On Glycolysis in Rat Eggs during Pre-Implantation Stages

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The experiments were undertaken to determine the metabolic pathway of glucose and pyruvate in the rat embryo during the preimplantation stages.

In the cleaved rat ova before blastocyst, the production of $^{14}CO_2$ from $^{14}C_1$-glucose is far more rapid than that produced from $^{14}C_6$-glucose, suggesting a very active pentose shunt during early cleavage stages.

In the blastocyst, the $^{14}C_1$ to $^{14}C_6$ ratio in the $^{14}CO_2$ production from both labelled glucoses is smaller than that of the ova before blastocyst formation.

In all stages of the rat embryos, the production of carbon dioxide from $^{14}C_1$-pyruvate was much greater than that of the labelled glucose.

The sum of the CO_2 production from both glucose and pyruvate accounted for 30 percent of the O_2 uptake utilized by the rat ova in all stages.

In all rat embryo cases the incorporation of the carbon from both glucoses into the TCA insoluble fraction of the embryo was much greater than that of pyruvate.

The roles of both glucose and pyruvate in the energy metabolism of rat embryo during early cleavage stage were discussed.

Metabolism in mammalian ova during the pre-implantation stage has been reviewed by several workers (1–4). Energy metabolism in mouse, rat and rabbit ova have been studied during recent years by various techniques. It has been reported that the metabolic pathways of glucose in the energy metabolism of ova during the cleavage stage were different according to the species. It has been shown that in rabbit ova before blastocysts formation, glucose was utilized via an active pentose shunt (5).

Brinster (6, 7) has shown that the magnitude of the C_1 to C_6 ratio in the mouse embryos did not indicate a high activity of the pentose shunt in comparison to the Embden-Meyerhof and Krebs Cycle.

However, there was no information on the metabolism of glucose in mammalian ova, estimating simultaneous uptake of O_2, carbon dioxide production, and accumulation of carbon from labelled glucose.

Also, the pathway of glucose oxidation in rat ova remains unknown.

Therefore, the purpose of this investigation is to determine the pathways of glucose metabolism. In the present report, O_2 uptake, carbon dioxide production by rat embryo and incorporation into the ova were calculated simultaneously, and these results were discussed in comparison to each other.

Materials and Methods

Rat Embryos: a) Unfertilized ova (1-cell stage) were collected from superovulated rats aged 25 to 29 days. b) Fertilized ova at 1-cell stage and blastocysts were collected on the first and fifth days of pregnancy from adult rats, which had been superovulated and mated, respectively.

Unfertilized and fertilized ova at the 1-cell stage were denuded of follicular cells by the hyaluronidase treatment. After remove of follicular cells, the ova were washed three or more times in Krebs-Ringer Phosphate Buffer (KRP): then they were moved in small droplets to a petri dish and the number of ova were counted for use in the final experimental run.

Incubation Systems: A sufficient number of embryos, depending on the developmental stage, were picked up from the droplets in as small number as possible (approximately 10 μl) with a finely drawn breaking pipette and placed in 40 μl of medium in the main chamber of the manometer (Fig. 1 total space is 2000–2400

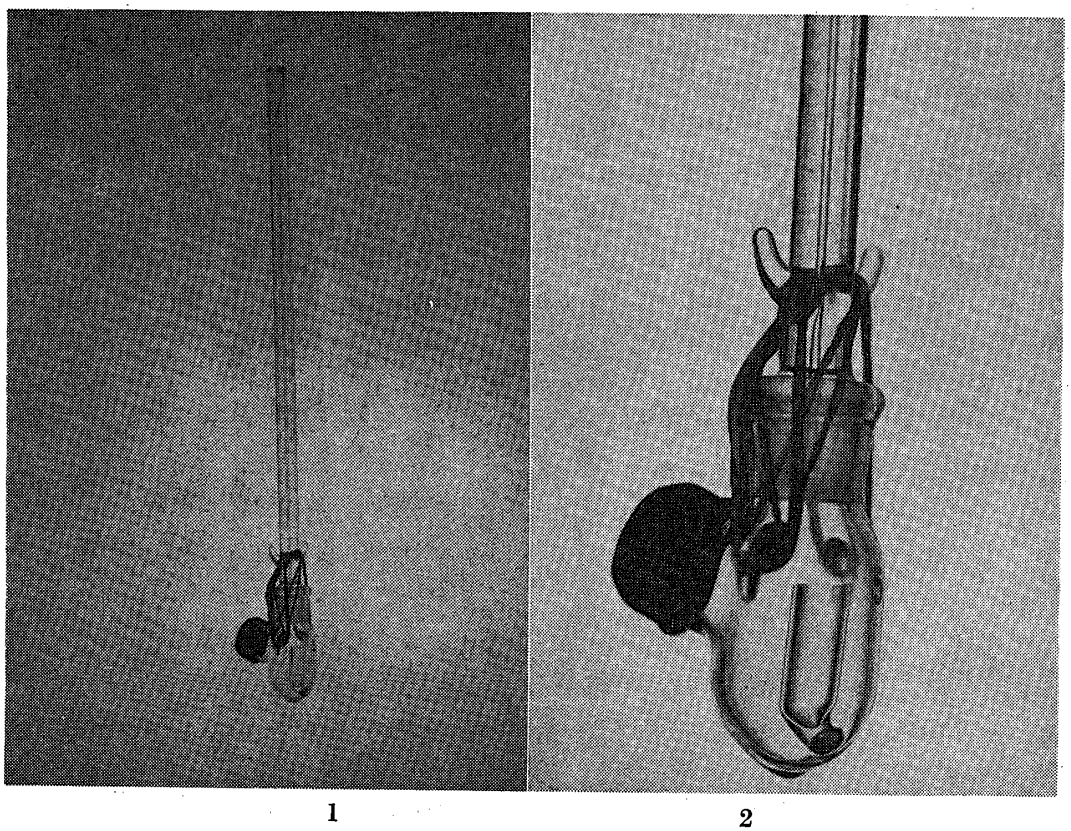

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FIG. 1 Manometer.

μl). Also 30 μl of 0.1 N NaOH had been placed in the side room. The number of embryos used varied from 400 per experimental run in the case of 1-cell and 2-cell ova to 50 per run for blastocyst. Then, 2 μCi of the radioactive glucose or pyruvate were added to the medium containing a sufficent number of embryos.

The manometer was plased in an incubator regulated at 38±0.01°C. After pre-incubation for 15 minutes, 8 μl of Brody solution were placed in the upper part of the manometer for the seal and fourth making of meniscus. Controls containing no embryos were incubated with each experiment. Also, the thermobalometer was simulutaneously activated.

The menisucs in the manometer was read at 30 minutes intervals for 4 hours.

Measurements of Radioactivity: At the end of 4 hrs, the top of the manomater was sealed with parafilm to prevent the loss of CO_2 gas and 100 μl of 0.1 N HCl were added to the main chamber containing the embryos to be revealed $^{14}CO_2$ retained in culture medum and to stop the reaction. This addition was made through the rubber stopper by means of a 20 gauge needle. The manometer was allowed to stand at room temperature for 24 hrs.

The labelled carbon dioxide which was trapped in side room by the 30 μl of 0.1 N NaOH was placed in scintillation vials and the side room is washed several times with distilled water: then the washed water is also placed in the vials. The vials were dried under an infrared lamp.

The recovery test of $^{14}CO_2$ using a standard sodium ^{14}C-bicarbonate in the above-mentioned method, shows that in 18 hrs 96 percent of the labelled CO_2 from the cultured medium is trapped in the 0.1 N NaOH.

The culture medium containing the embryos is 0.5 ml of cold trichloracetic acid (TCA) and the embryos were glass filterated and the filterates washed three or more times with TCA.

The radioactivity was measured with the toluene-PPO-POPOP system in an Aloka, liquid scintillation spectrometer, using the full spectum window at ambient temperature.

The counting efficiency for ^{14}C was 86–92 percent.

Anerobic Glycolysis: The production of carbon dioxide from the labelled glucose under anerobic conditions was estimated. The anerobic condition was produced by flushing with 100% N_2 gas for approximately 3 minutes.

All of the experiments were conducted according to the above-mentioned method.

Results

$^{14}CO_2$ Production from Glucose Lavelled in Positions C_1 and C_6: In the experiments, an attempt was made to estimate the activity of the pentose shunt by employing glucose labelled in the C_1 position (specific activity 4.79 mCi/mM).

The results of these experiments are shown in Table 1.

TABLE 1. *$^{14}CO_2$ Produced from Glucose Labelled in Positions C_1 and C_6*

Stage of development	Glucose *DPM/ovum/hr.*		Ratio of C_1 to C_6
	C_1	C_6	
1-cell (unfertilized)	155.3±50.2 (4)	21.3±5.6 (4)	7.29
1-cell (fertilized)	250.0±48.3 (3)	19.2±6.8 (3)	13.02
2-cell	213.2±48.2 (4)	26.1±3.4 (4)	8.17
Blastocyst	1336.0±215.8 (4)	548.5±98.4 (5)	2.43

Parenthesis are number of experiments

In all cases the production of carbon dioxide from $^{14}C_1$-glucose was greater than that from $^{14}C_6$-glucose.

In the cases of 1-cell and 2-cell ova, the production of carbon dioxide from glucose labelled in positions C_1 and the C_6 did not differ in level. The sudden increase in CO_2 production from $^{14}C_1$- and $^{14}C_6$-glucose occurred in blastocysts stage. The value of $^{14}CO_2$ production from both C_1 and C_6 glucose increased 5 and 20 fold respectively in comparison to that estimated in the 2-cell ova.

The C_1 to C_6 ratio in unfertilized and 2-cell ova was about same the value, but the ratio in the case of fertilized ova was greater than that recorded for either the unfertilized or the 2-cell ova. The ratio of C_1 to C_6 was 2.43.

Effect of Pyruvate on CO_2 Production from the Labelled Glucose: As shown in Table 2, the production of carbon dioxide from both $^{14}C_1$ and $^{14}C_6$-glucose in the cases of 1-cell to 2-cell ova increased from 10 to 67 percent in the presence of cold pyruvate. In blastocysts, carbon dioxide produced from the labelled glucose decreased 15.2–23.8 percent (Table 2).

O_2 Uptake and Consumed Glucose: The results are given in Table 3. Oxygen consumption of all cases in the presence of labelled glucose did not differ according to the position of the labelled carbon. The value of O_2 uptake in rat ova was

TABLE 2. *Effect of Pyruvate on $^{14}CO_2$ Production from Glucose Labelled in C_1 and C_6*

Stage of development	Pyruvate concentration mol.	Glucose *DPM/ovum/hr.*		Ratio of	Percent of decreased	
		C_1	C_6	C_1 to C_6	C_1	C_6
1-cell (unfertilized)	2.5×10^{-3}	170.0±50.2	29.9±8.9	5.6	109.6	140.4
2-cell	〃	358.3±85.3	37.0±13.4	9.6	167.9	141.8
Blastocysts	〃	500.3±98.4	130.4±42.3	3.8	15.2	23.8

TABLE 3. *O_2 Uptake and Consumed Glucose Labelled in Positions $^{14}C_1$ and $^{14}C_6$*

Stage of development	O_2 uptake $\mu\mu$ *mol/ovum/hr.*		Glucose $\mu\mu$ *ml/ovum/hr.*	
	C_1	C_6	C_1	C_6
1-cell (unfertilized)	0.53±0.10	0.52±0.23	0.014±0.003	0.0029±0.0006
1-cell (fertilized)	1.20±0.42	0.89±0.26	0.022±0.005	0.0074±0.004
2-cell	0.65±0.23	0.72±0.19	0.019±0.002	0.0016±0.0003
Blastocysts	2.46±0.38	2.99±0.52	0.120±0.067	0.035±0.005

similar to that measured by one of the authors previously (8), using the techniques of Catesian diver manometery.

The ratio of consumed glucose to O_2 uptake in $^{14}C_1$-glucose was 0.05 to 0.02.

$^{14}CO_2$ Production from $^{14}C_1$-Pyruvate: In general, the counts (CPM) in the controls containing no embryos were between 1,000 and 2,000. These values were 10 to 20 fold greater than that which resulted spontaneously from the labelled glucose in the control.

In all cases the production of carbon dioxide from $^{14}C_1$-pyruvate was much greater than that of the labelled glucose (Table 4).

The 1-cell stage unfertilized ova utilize more pyruvate in comparison to the fertilized ones. Also, the activity in the carbon dioxide production of unfertilized ova was not different to that of the blastocyst stage.

TABLE 4. *$^{14}CO_2$ Production from Pyruvate Labelled in Position C_1*

Stage of development	No. of Analysis	CO_2 production *DPM/ovum/hr.*	Ratio of Pyruvate to G-1
1-cell (unfertilized)	4	3090.0±350	19.90
glucose	3	2342.9±285	15.08
1-cell (fertilized)	3	2441.7±465	9.76
2-cell	3	1465.5±125	6.87
glucose	3	1732.5±250	8.12
Blastocysts	4	4396.7±478	3.20
glucose	3	4890.5±245	3.66

The presence of cold glucose did not significantly affect the production of carbon dioxide other than in the case of the unfertilized ova.

The ratio of $^{14}C_1$-pyruvate to $^{14}C_1$-glucose in $^{14}CO_2$ production from their oxidation was in the range from 3.2 to 19.9. These ratios decreased in progress with the cleavage stage.

TABLE 5. *The Relationship between O_2 Uptake and Consumed Pyruvate-1-^{14}C*

Stage of developent	O_2 uptake $\mu\mu$ *mol.*	Pyruvate $\mu\mu$ *mol.*
1-cell (unfertilized)	0.86±0.23	0.210±0.048
1-cell (fertilized)	1.2 ±0.43	0.242±0.041
2-cell	1.29±0.32	0.170±0.053
Blastocyst	1.96±0.48	0.117±0.038
glucose added	2.82±0.61	0.13 ±0.048

O_2 Uptake and Consumed $^{14}C_1$-Pyruvate: As shown in Table 5, the O_2 uptake and consumed $^{14}C_1$-pyruvate increased progressively during the cleavage stages. However, the increase in rate of O_2 uptake in the progress of the development was greater than that of the consumed pyruvate.

The Ratio of Oxidized $^{14}C_1$- and $^{14}C_6$-Glucose and $^{14}C_1$-Pyruvate to O_2 Uptake: The results are given in Table 6. In the 1-cell stage, the oxidation of $^{14}C_1$-glucose accounted for 16 percent of the oxygen utilized by the embryo.

The oxidation of $^{14}C_1$-glucose increased during the cleavage stage and the utilized $^{14}C_1$-glucose accounted for 42.1 percent of the oxygen consumed by blastocysts. $^{14}C_6$-glucose oxidation was 6.3 to 12.0 percent of the oxygen utilized by the embryo.

At blastocyst stage, the amount of oxidized $^{14}C_1$-glucose decreased in the presence of cold pyruvate.

On the other hand, $^{14}C_1$-pyruvate was actively oxidized by the embryo at all stages in comparison to that of glucose. The amount of oxidized $^{14}C_1$-pyruvate was in the range from 65.0 to 77.2 percent of the oxygen utilized by the embryo at all stages.

Incorporation of ^{14}C-Glucose and Pyruvate into TCA Insoluble Fraction of Embryo: It was found that the radioisotope from $^{14}C_1$- and $^{14}C_6$-glucose or $^{14}C_1$-

TABLE 6. *Ratio of Consumed Glucose-1-^{14}C, G-6-^{14}C and Pyruvate per O_2 Uptake*

Stage of development	Addition of pyruvate	Glucose %		Pyruvate %	
		C_1	C_2		
1-cell (unfertilized)	—	16.0	6.3	—	77.2
	P	16.7	—	G	55.7
1-cell (fertilized)	—	19.3	8.3		63.1
2-cell	—	30.1	2.3	—	56.3
	P	40.8	8.7	G	66.4
Blastocyst		42.1	12.0	—	63.4
	P	18.8	2.3	G	45.2

The values are estimated from the data, assuming each carbon composed a substrates could be oxidized to equal rate

TABLE 7. *Incorporation of ^{14}C from Glucose and Pyruvate into TCA Insoluble Fraction of Rat Embryos*

Stage of development	Glucose *DPM/ovum/hr.*		Ratio of C_1 to C_6	Pyruvate *DPM/ovum/hr.*	Ratio of P-G_1	
	C_1	C_6			G-C_1	G-C_6
1-cell (unfertilized)	754.3±120.4	255.5±34.5	2.95	203.6±98.1	0.27	0.797
1-cell (fertilized)	81.1±18.5	55.4±14.5	1.46	115.7±62.4	1.42	2.08
2-cell	519.8±58.6	25.5±11.2	20.63	60.7±20.	0.117	2.38
Blastocysts	3283.3±523	2549.9±452.0	1.288	173.1±110.	0.053	0.068

pyruvate accumulated in the TCA insoluble fraction of the embryos after 4 hours of incubation.

Radioactivity from the ^{14}C-glucose was detected in the TCA insoluble fraction of the embryos at all stages, suggesting incorporation in nucleic acids and the protein fraction (Table 7). In all stages of the embryos, the incorporated radioactivity from $^{14}C_1$-glucose was greater than that of glucose labelled in position of C_6. The incorporation of both labelled glucoses into the TCA insoluble fraction was 2.6 to 11-fold higher than the activity of the carbon dioxide production from them.

The incorporation of $^{14}C_1$-pyruvate into TCA insoluble fraction was much smaller than that of both labelled C_1 and C_6 glucoses. The radioactivities from pyruvate were from one fourth to one twelfth that from labelled glucose (Table 7).

Anerobic Oxidation of Labelled Glucose and Pyruvate: Under anerobic conditions, the production of carbon dioxide from $^{14}C_1$- and $^{14}C_6$-glucose decreassed to about 48 percent of the amount of $^{14}CO_2$ produced aerobically from both glucoses (Table 8).

Especially, $^{14}CO_2$ production in blastocysts decreased about one fourth (Fig. 2).

The decrease in the incorporation of the labelled substances into the TCA insoluble fraction was small in comparison to $^{14}CO_2$ production from each of the labelled substances.

TABLE 8. *$^{14}CO_2$ Production from G-1-^{14}C, G-6-^{14}C and P-1-^{14}C under Anerobic Conditions*

Stage of development	$^{14}CO_2$ production *DPM/ovum/hr.*		
	G-*1*-$^{14}C^a$	G-*6*-$^{14}C^b$	P-*1*-$^{14}C^c$
1-cell	66.9±10.3	9.3±1.3	—
2-cell	94.3±13.1	13.3±3.2	—
Blastocysts	242.2±61.2	51.2±8.1	2764.3±710.2

Notes: a, b and c are glucose-1-^{14}C, glucose-6-^{14}C and pyruvate-1-^{14}C.

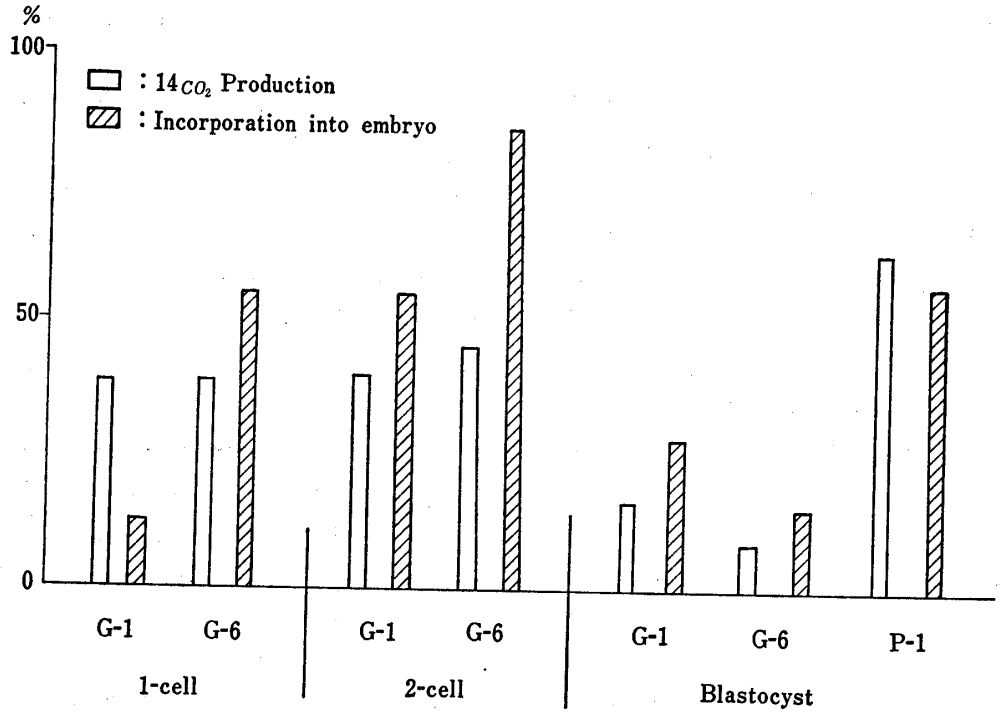

FIG. 2 Percent of anerobic to aerobic metabolism

Discussion

In the early developmental stage, if any glucose is oxidized via the Embden-Meyerhof route, $^{14}C_1$- and $^{14}C_6$-glucose must be oxidized at the same rate.

In the rabbit embryos, the $^{14}C_1$ to $^{14}C_6$ ratio in the $^{14}CO_2$ production from both labelled glucoses is high before blastocyst formation and this ratio is close to 1.0 following blastocysts. These facts would suggest a very active pentose shunt during the early cleavage stages and the Embden-Meyerhof route following blastocysts formation (5). Brinster has shown in the mouse, that the magnitude of the $^{14}C_1$ to $^{14}C_6$ ratio does not indicate a high activity of the pentose shunt in comparison to Krebs cycle and that the ratio in the mouse changes very little during the pre-implantation stages.

In this experiment, however, the production of $^{14}CO_2$ from $^{14}C_1$-glucose is far more rapid than that produced from $^{14}C_6$-one and the magnitude of the $^{14}C_1$ to $^{14}C_6$ ratio is very high before blastocyst formation. These facts might suggest the active conversion of glucose to pentose before blastocyst formation. Also, unlike the rabbit and mouse embryos the ratio in the rat following blastocyst does not fall to 1.0.

In the rat embryo before blastocysts, the production of carbon dioxide from the labelled glucose is affected by the presence of pyruvate. This may suggest that glucose could be utilized in the constant level for energy metabolism of rat embryo before blastocysts.

Recent investigations on the embryo of mouse and rabbit indicate that pyru-

vate is oxidized to a significantly greater degree than glucose at all stage of pre-implantation (4, 6, 7).

In this experiment, it was recognized that the rat embryos can very easily oxidize pyruvate to CO_2 at all stages of early development.

The magnitude of oxygen uptake estimated by the semimicro manometer in this experiment was about the same as that measured previously by the author (8, 9), using a ultramicromanometer e.g. Catesian diver method.

The amount of CO_2 produced from labelled substances by the embryos can compare to toal oxygen uptaken by the same stage of ova.

Brinster has shown that at the times after ovulation, the amount of CO_2 produced from pyruvate would acount for 100 percent of the oxygen uptake.

Assuming each carbons composing a labelled substances (e.g. C_1 to C_6 in the case of glucose and C_1 of pyruvate) can be oxidized at an equal rate by rat embryos, the amount of CO_2 produced from $^{14}C_1$-pyruvate could correspond to 55–77 percent of the oxygen consumed by the rat embryo at all stages of pre-implantation.

The percentage of oxygen which can be accounted for by the production of CO_2 from labelled glucose increases from 16 percent at the 1-cell stage to 43 percent at the blastocyst stage.

This indicates an increased ability of the embryo to oxidize glucose.

In all stages of rat embryos, the sum of the amount of CO_2 from labelled glucose and pyruvate could correspond to 100 percent of the total oxygen utilized by the same embryos.

As mentioned above, however, it is the fact that the carbon in all positions of the substrates did not oxidize equally. Therefore, the sum of oxidation of C_1- and C_6-glucose and C_1-pyruvate could only account for 30 percent of the oxygen uptaken by the rat embryos during the cleavage stage. Also, the energy required for the development during these stages should be obtained from oxidation of endogenous and other substrates.

In this experiment, the incorporation of glucose carbon labelled in position of C_1 and C_6 into TCA insoluble fraction of the rat embryos is much greater than the formation of $^{14}CO_2$ from each of glucose for all developmental stages.

Also, these results may suggest that the rat ova, as in mouse, rabbit and monkey could utilize pyruvate as an energy source than the precursor in synthesetic process during the cleavage stage.

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