DIFFERENT PATTERNS OF ENERGY METABOLISM IN THE RAT AND MOUSE ZYGOTE

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Summary. The development of rat zygotes *in vitro* to the two-cell stage occurred if lactate, phosphoenolpyruvate (PEP), pyruvate or oxaloacetate were present in the media. When rat and mouse zygotes were cultured in the same droplet of medium containing lactate or PEP, mouse zygotes did not develop to the two-cell stage but the rat zygotes cleaved.

Techniques for the culture of preimplantation mouse embryos have made it possible to clarify the nutritional requirements of the earliest cleavage divisions in this species. The cleavage of the zygote can only take place *in vitro* when pyruvate or oxaloacetate are present in the culture medium (Biggers, Whittingham & Donahue, 1967; Whittingham, 1969). Development between the two- and eight-cell stages is supported by lactate, pyruvate, phosphoenolpyruvate (PEP) and oxaloacetate (Brinster, 1965a). This investigation of the energy requirements of the rat zygote was undertaken to determine if the results so far reported for the early mouse embryo are species-specific or are equally relevant for the cleavage stages of other mammalian species. The basic medium for the studies was a Krebs-Ringer bicarbonate solution, modified according to Biggers *et al.* (1967), with a pH of 7.2 to 7.4 and an osmolarity of 308 mosmol. The embryos were cultured according to the method of Brinster (1963) in 5% CO₂ in air under paraffin oil (Uvasol: Merck A.G. Darmstadt, Germany) in tissue culture dishes (Falcon Plastics: Oxnard, California, U.S.A.) or without oil in the U-shaped wells of Cooke-Mikrotiter testplates (C. A. Greiner, Nürtingen, Germany). Wistar rats (strain SW 72: Winkelmann, Kirchborchen, Germany) were bred under a reversed day/night cycle (dark period from 24.00 to 12.00 hours). Mice (strain NMRI: Schwenke & Co., Nauheim, Germany) were also kept in an artificial day/night cycle (dark period from 20.00 to 08.00 hours). Females were placed with males for 3 hr (06.00 to 09.00 hours), and pregnant animals were killed at 16.00 hours (mice) or 18.00 hours (rats). Mouse zygotes were collected and the cumulus cells were removed with 150 units (mice) or 400 units (rats) hyaluronidase (Boehringer Mannheim, Germany)/ml according to the method of Biggers *et al.* (1967). Hyaluronidase can induce parthenogenetic cleavage (Graham, 1970) but even with a dose of 400 units hyaluronidase/ml unfertilized rat eggs did not cleave in media in which fertilized eggs developed to the two-cell stage. The

number which had undergone first cleavage division in culture was checked after 24 hr for the mouse and after 36 hr for the rat embryos.

Rat embryos were cultured for 36 hr in media containing one of the following energy sources (range of concentrations tested in parentheses): lactate (0.02 to 25 mM), pyruvate (0.02 to 2.0 mM), oxaloacetate (0.02 to 2.0 mM), PEP (0.02 to 25 mM), acetate (0.02 to 2.0 mM), malate (0.02 to 2.0 mM), α -keto-glutarate (0.05 to 5.0 mM), citrate (0.02 to 20 mM), 2-phosphoglycerate (0.05 to 5.0 mM), glucose (0.5 to 5.0 mg/ml). Rat zygotes cleaved in media containing pyruvate, oxaloacetate, lactate or PEP. In pyruvate and oxaloacetate, the highest rate of development of rat zygotes occurred at concentrations similar to those tested by Biggers *et al.* (1967) with mouse embryos. The cleavage of rat zygotes in lactate and PEP contrasts with the findings so far reported for the mouse zygote. The cleavage of rat zygotes in these four substrates is shown in Table 1.

Table 1. Cleavage of rat zygotes *in vitro* in different concentrations of pyruvate, oxaloacetate, lactate and phosphoenolpyruvate

Concentration (mM)	Pyruvate	Oxaloacetate	Lactate	Phosphoenol-pyruvate
25.00	—	—	87 $\pm$ 15 (5)	78 $\pm$ 13 (5)
2.00	82 $\pm$ 10 (5)	78 $\pm$ 12 (5)	86 $\pm$ 12 (5)	90 $\pm$ 8 (6)
0.20	89 $\pm$ 8 (10)	85 $\pm$ 6 (8)	*17 $\pm$ 8 (6)	*33 $\pm$ 10 (5)
0.02	*15 $\pm$ 5 (5)	*10 $\pm$ 5 (6)	0 (5)	0 (4)

The values are the mean percentage of a treatment replicated the number of times shown in parentheses. Twenty zygotes were used in each experiment.

* Significantly different from means at higher concentrations, $P < 0.001$.

The rat zygote uses the same four compounds for its development as does the two-cell mouse embryo. Mouse zygotes, however, did not cleave in media containing lactate or PEP as the only energy source. Further data were obtained by incubating rat and mouse zygotes in the same droplet. Since first cleavage occurs earlier in the mouse than in the rat under the present experimental conditions, it was possible to observe the development of the zygotes of the two species after different periods of time (Table 2).

The pyruvate data served as controls. The experiments, using concentrations of 2 mM-lactate and 2 mM-PEP, showed that the numbers of cleaving embryos were never more than the numbers of rat zygotes used in the experiments. Identical results were obtained at a concentration level of 25 mM for both of these substrates. We hoped to prove that the cleaved and the uncleaved embryos belonged to the two different species on the basis of differences in the lactate dehydrogenase (LDH) isoenzyme pattern, since both rat and mouse pre-implantation embryos only contain LDH-1 (Brinster, 1965b; Epstein, Kwok & Smith, 1971; Engel & Petzoldt, 1973). At different acrylamide concentrations (5% and 7.5%) as well as at different pH values of the electrode buffer (pH 8.1 and 9.2), however, electrophoresis with adult tissues revealed that although LDH-2 to -5 isoenzymes of the two species could be separated from one another on acrylamide gels, the LDH-1 isoenzymes could not. The LDH-isoenzyme pattern could not, therefore, prove that the two-cell embryos belonged to one

Table 2. The development *in vitro* of forty rat zygotes and thirty-six mouse zygotes during combined incubation

Incubation time (hr)	Energy substrate and no. of embryos									
	0.2 mM-Pyruvate				2.0 mM-Phosphoenolpyruvate					
	One-cell		Two-cell		Degenerated	One-cell		Two-cell		Degenerated
0	76	0	0	0	0	76	0	0	0	0
24	39	34	3	3	0	53	23	5	13	13
36	3	69	4	4	22	16	38	36	15	15
60	2	68	6	6	24	14	38	35	18	18

The data for each substrate are the summary of four single experiments carried out with ten rat zygotes and nine mouse zygotes.

species and the one-cell embryos and degenerated embryos to the other. The inability to culture rat zygotes beyond the two-cell stage even when they were transferred to standard culture media agrees with the results of Biggers *et al.* (1967) on the development of mouse zygotes *in vitro*. Mouse zygotes are known to be unable to convert lactate to pyruvate despite their high LDH activity (Brinster, 1965b). Zeilmaker, Hulsman, Wensinck & Verhamme (1972) and Sorensen (1972) assumed that the NAD concentration in mouse zygotes was very low because they cleaved successfully *in vitro* in the presence of lactate and NAD. The same hypothesis has recently been supported by Cross & Brinster (1973). Our data for the rat make the interpretation of this problem more difficult since the LDH activity in preimplantation rat embryos is much lower than in mouse embryos at the same developmental stage (Brinster, 1967).

The viability of our two-cell rat embryos produced *in vitro* was not known. Toyoda & Chang (1974) have cultured two-cell rat embryos from one-cell embryos fertilized *in vitro*, but they did not develop beyond the two-cell stage. When the two-cell embryos were transferred to the oviducts of pseudopregnant females, however, normal embryos were obtained at term. It seems possible, therefore, that our two-cell rat embryos were also viable. In the mouse, oocyte maturation and the first cleavage division are supported by oxaloacetate and pyruvate. Further investigations are necessary to determine the energy requirements for oocyte maturation in the rat.

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