THE LACTATE DEHYDROGENASE IN PREIMPLANTATION MOUSE EMBRYOS OF QUACKENBUSH AND SWISS MICE

R.L. BRINSTER

Laboratory of Reproductive Physiology, School of Veterinary Medicine
University of Pennsylvania, Philadelphia, Pennsylvania 19104, USA

Received 22 July 1971

1. Introduction

The lactate dehydrogenase (EC.1.1.1.27) activity of the newly ovulated mouse ovum is extremely high, about ten times the activity found in skeletal muscle, which is the adult tissue with the highest activity [1]. It has been suggested that this high activity might be related to the unusual dependence of the ovum and early embryonic stages of development on pyruvate and lactate for energy sources [1, 2]. Isozyme studies by Auerbach and Brinster demonstrated that the lactate dehydrogenase (LDH) was almost exclusively composed of B subunits and that the isozyme pattern changed to one with predominantly A subunits at the time of implantation [3]. Rapola and Koskimies confirmed the isozyme pattern and also demonstrated the predominance of B subunits in early cleavage stages using biochemical techniques [4].

Recently, Gibson and Masters reported that the Quackenbush strain of mice in Australia has an isozyme pattern containing predominantly A subunits [5]. They suggest that the B subunits are adsorbed onto the surface of the ovum from the oviducal fluid after ovulation. This seems an unlikely explanation of the predominance of the B subunits in the Swiss strain since Brinster found that the level of LDH activity in the pre-ovulatory oocyte was the same as the activity of the early cleavage stage ova taken from the oviduct [6]. Epstein et al. have recently confirmed this similarity in activities [7]. In Gibson and Masters' studies the isozyme pattern and total activity of LDH varied considerably depending on the *in vitro* handling method, but in all cases the total LDH activity was 5% or less of that found in Swiss strain ova [1, 5]. They felt the variation in pattern and activity was a result of removal of adsorbed B type subunits. Using the same techniques as Gibson and Masters used on Quackenbush ova, Epstein et al. were unable to demonstrate any change in activity of the ova from Swiss mice [7].

The evidence to date suggests that a difference in the LDH activity and isozyme pattern exists between the Quackenbush and Swiss strain of mice or that differences in techniques account for discrepancies between the findings. If indeed there is a difference in the LDH between the strains, it would prove a powerful tool to study gene action in the early mammalian embryo. Since a clearcut answer to this important question can only be obtained by simultaneous studies on the two strains, we imported the Quackenbush strain from Australia and report here the results of the studies on the two strains.

2. Materials and methods

Ovulated ova and cleavage stage embryos were obtained from both Swiss and Quackenbush strain mice at specific times after ovulation. In most cases, the females were superovulated by the injection of 5 I.U. of pregnant mare serum (Gestyl, Organon), followed 48 hr later by the injection of 5 I.U. of human chorionic gonadotrophin [2]. Cleavage stage

Table 1
Lactate dehydrogenase activity in Swiss and Quackenbush two-cell ova.

Strain	Number of times centrifuged			
	0	1	3	4
Swiss	5.22 ± 0.30	5.24 ± 0.22	4.88 ± 0.27	5.14 ± 0.22
Quackenbush	5.51 ± 0.24	5.37 ± 0.28	5.32 ± 0.24	5.72 ± 0.30

Activities are in moles × 10^{-8} per ovum per hr. The values are means of six determinations ± SEM. See text for details of centrifugation.

embryos were flushed from the reproductive tract [2], oocytes were dissected from the ovary [6], and 9-day embryos were dissected from the uterus [3]. All manipulations of the embryo were done in Brinsters's Medium for Ovum Culture-2 (BMOC-2), which is similar in salt composition to Krebs Ringer bicarbonate and contains 1 mg/ml of crystalline bovine serum albumin [2, 8]. The embryos were washed three times by centrifugation (1000 *g*, 3 min) through 10 ml of BMOC-2 followed by one centrifugation (1000 *g*, 3 min) through 10 ml of 0.9% saline.

After collection and washing, ova were placed in 6 × 60 mm tubes (1 per tube or 20 per tube) and stored at -70°. For total LDH activity measurements, tubes containing one ovum were placed in ice, and reaction mixture consisting of 100 μl of 0.1 M sodium phosphate buffer (pH 7.5) containing pyruvate (1 mM), NADH (1 mM) and crystalline bovine serum albumin (0.05%) was added to the tube. The tubes were immediately placed in a water bath at 37° for 15 min. At the end of the incubation the tubes were placed in boiling water for two minutes to stop the enzyme reaction. The quantity of NAD formed was measured fluorometrically using a modification of the strong alkali procedure of Lowry et al. [9]. To the 100 μl of reaction mixture was added 50 μl of 1N HCl followed by gentle agitation of the tube to destroy excess NADH. After two minutes, 250 μl of 9 N NaOH was added to the tube, the contents, the contents mixed, and heated for fifteen minutes at 60°. The fluorescence of an aliquot of the contents of the tube was measured in 5 ml of water in a Turner Model 111 fluorometer. Appropriate standards and blanks were carried through the entire procedure.

The isozyme pattern was determined using vertical starch gel electrophoresis [10]. The electrode buffer was 0.233 M Tris and 0.086 M citric acid with a pH of 7.0. The gel was 10.4% starch in a 1:35 dilution of the electrode buffer. The samples of embryos (20 per tube) were placed in the gel wells and the starch was run for four hours in a voltage gradient of 6 V/cm with the anode at the bottom. Samples of heart and skeletal muscle were used as markers of electrophoretic migration. The gel was sliced after electrophoresis, and the LDH bands were developed by incubating the starch for two hours at 37° in a mixture consisting of 0.5 M Tris, pH 7.1 (15 ml), 1.0 M sodium lactate (10 ml), 0.1 M NaCN (5 ml), water (70 ml), NAD (50 mg), Nitro Blue Tetrazolium (30 mg) and Phenazine Methosulfate (2 mg).

3. Results and discussion

Two-cell embryos were collected from Swiss and Quackenbush mice simultaneously and were washed four times by centrifugation as described above. Embryos were placed in tubes for total LDH activity measurements before centrifugation and after the first, third and fourth centrifugations. The results of the activity measurements made on these embryos are shown in table 1. There is very little difference in the total activity between the strains. In addition, an analysis of variance of the data indicated that there was no significant effect of centrifugation on LDH activity in either the Swiss or Quackenbush strain. Neither three washes in BMOC-2 nor one wash through saline decreased the activity. These findings are in sharp contrast to the results of Gibson and Masters [5] who found that Quacken-

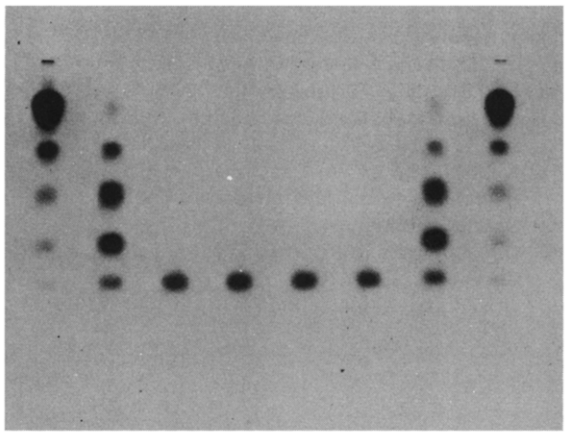

Fig. 1. From left to right the wells contained Swiss skeletal muscle, Swiss heart muscle, 20 Swiss unfertilized ova, 20 Swiss two-cell ova, 20 Quackenbush two-cell ova, 20 Quackenbush unfertilized ova, Quackenbush heart muscle, and Quackenbush skeletal muscle. The direction of migration was from top to bottom, and the bottom was positive. From top to bottom the LDH bands are A_4, A_3B, A_2B_2, AB_3 and B_4.

bush ova had a low LDH activity and that washing Quackenbush ova by centrifugation resulted in a sharp fall in total activity. The results of washing Swiss ova by centrifugation are in agreement with those recently reported by Epstein et al. [7]. The data shown in table 1 suggest strongly that the low LDH activities found by Gibson and Masters result from some characteristic of their techniques in handling the ova.

Several LDH determinations made on Quackenbush embryos just prior to implantation indicated that the LDH activity had decreased to about one tenth (5.72×10^{-9} mole/embryo/hr) the activity found at ovulation. In addition, the oocytes of the Quackenbush strain have an LDH activity similar to the two-cell ova, thus suggesting that very little LDH is adsorbed from the oviduct. These findings are similar to the results for Swiss mice [1, 6] and lend additional credence to the belief that the strains are similar in their LDH activities during development.

After the fourth wash by centrifugation, groups of 20 two-cell ova were placed in tubes and frozen for electrophoresis. These embryos were then thawed and the LDH isozyme pattern determined as described above. Fig. 1 shows a comparison of Swiss and Quackenbush two-cell and unfertilized ova. There is no difference between the strains or the developmental stages in the migration of the enzyme from the ova. The LDH is almost exclusively composed of B subunits. Fluid from the centrifugation tubes was also collected and 2 ml was freeze-dried and the protein resuspended in 50 μl. No electrophoretic LDH isozyme pattern could be detected in either the original fluid or in the resuspended freeze-dried protein. There was no evidence that either B or A subunits had been washed off the ova of either strain. The results of these studies are in marked contrast to those of Gibson and Masters who found that two-cell Quackenbush embryos contained mostly A subunits of LDH and felt that the B subunits were adsorbed and could be washed off.

Other electrophoretic assays of Quackenbush embryos demonstrated that the LDH isozyme pattern remained predominantly B subunits until implantation on day 5. However, by day 9 there was a great preponderance of A subunits. Thus the isozyme pattern of Quackenbush mice and the changes in the pattern are similar to those previously described for Swiss mice [3].

It is not possible to give a precise reason why the results of this study are different from those of Gibson and Masters. Perhaps the techniques Gibson and Masters used in handling the embryos resulted in damage to the cell membranes and subsequent leakage of the enzyme. This would suggest the interesting possibility that the A subunits have a different intracellular location or binding than the B subunits since they found the B subunits were lost first. The important conclusion of the experiments reported here is that the LDH total activity and isozyme pattern of the preimplantation embryos of Quackenbush and Swiss strain mice are very similar or identical. This information should caution against the initiation of experiments to exploit in genetic or developmental studies the previously suggested difference in LDH activity and isozyme pattern.

Acknowledgements

I thank Dr. R.G. Wales, University of Sydney, Sydney, N.S.W., Australia, for the Quackenbush

mice. The expert technical assistance of Mrs. Margaret Van Meter is gratefully acknowledged. Financial support for this work was from PHS Research Grant No. 03071 from the National Institute of Child Health and Human Development.

References

[1] R.L. Brinster, Biochim. Biophys. Acta 110 (1965) 439.
[2] R.L. Brinster, J. Exp. Zool. 158 (1965) 59.
[3] S. Auerbach and R.L. Brinster, Exp. Cell Res. 46 (1967) 89.
[4] J. Rapola and O. Koskimies, Science 157 (1967) 1311.
[5] C. Gibson and C.J. Masters, FEBS Letters 7 (1970) 277.
[6] R.L. Brinster, J. Reprod. Fertility 17 (1968) 139.
[7] C.J. Epstein, L. Kwok and S. Smith, FEBS Letters 13 (1971) 45.
[8] R.L. Brinster, in: The Mammalian Oviduct, eds., E.S.E. Hafez and R. Blandau (University of Chicago Press, Chicago, 1969) pp. 419–444.
[9] O.H. Lowry, N.R. Roberts and J.I. Kapphahn, J. Biol. Chem. 224 (1957) 1047.
[10] O. Smithies, Advan. in Protein Chem. 14 (1959) 65.