THE SOURCE OF LACTATE DEHYDROGENASE IN PREIMPLANTATION MOUSE EMBRYOS

Charles J. EPSTEIN, Lillian KWOK and Sandra SMITH
Department of Pediatrics, University of California, San Francisco, California 94122, USA

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1. Introduction

Studies of enzyme activities in preimplantation mouse embryos of the Swiss-Webster strain have demonstrated several developmental patterns of change [1, 2]. Among these is the pattern manifested by lactate dehydrogenase (EC.1.1.1.27) and glucose 6-phosphate dehydrogenase (EC.1.1.1.49). The activities of these enzymes remain constant for the first two days of development, up to the 8–16 cell stage. There is then an exponential decline in activity over the next two days, and the activities in the late blastocysts are only about 15–20% of those in the early stages of development. The cause of the decline in activity is not known, but one possibility is the existence of specific processes leading to enzyme degradation or inactivation [1]. However, Gibson and Masters have recently suggested, on the basis of work with the Quackenbush strain, that the high activity of lactate dehydrogenase and the developmental decline in activity detected in mouse ova do not reflect the true enzymatic situation of the embryo [3]. Rather, they proposed that virtually all of the activity detected is the product of enzyme adsorbed from the oviducal fluid, and the decline in activity is attributed to loss of this surface enzyme secondary to changes in the surface and location of the embryo. Because of the obvious importance of these suggestions for an understanding of the regulation of enzyme concentrations in early mammalian embryos, the activity of lactate dehydrogenase in embryos from Swiss mice has been reinvestigated. The results obtained are not compatible with the hypothesis that the enzyme being assayed is adsorbed from the oviducal fluid.

2. Materials and methods

Mouse embryos ranging from 2 cells (day 1) to the late blastocysts stage (day 4) were obtained from random bred Swiss mice by the methods previously described [1]. All flushing of the oviducts and handling of the embryos was carried out in the culture medium of Whitten and Biggers [4]. Follicular oocytes were obtained by pricking follicles of ovaries removed two days after the unmated animals received the priming dose of pregnant mare serum. Embryo culture and enzyme assay were carried out as previously described [1, 2]. Electrophoresis in 7.5% polyacrylamide gels was carried out at pH 8.1 with a continuous tris-glycine buffer system. The stacking gel was omitted. The gels were stained for lactate dehydrogenase activity with a mixture consisting of 1.6 g Na-D, L-lactate, 10 mg NAD, 0.8 mg phenazine methosulfate, and 10 mg nitro blue tetrazolium in 40 ml of 0.05 M tris-HCl, pH 7.1.

3. Results and discussion

The lactate dehydrogenase activity of 2 cell embryos from Swiss mice was 40.2 ± 1.1 (S.E.) nmoles/hr/embryo at 37°. This is similar to the values previously observed by us and by Brinster [1, 5]. If it is assumed that the protein content of a 2 cell embryo is about 26 ng [6], this activity corresponds to about 26 μmoles/min/mg protein at 37°. Gibson and Masters [3] reported a value of 0.2 to 0.8 I.U. (or μmoles/min) per mg protein. Even taking into account the fact that their lower assay temperature would reduce the measured activity by 2–3 fold,

their highest value is still at least 10-fold lower than that reported here. Also, in contrast to their results, we observed no lactate dehydrogenase activity in the fluid in which the embryos were suspended.

The activity of lactate dehydrogenase was not affected by washing the embryos by transferring them three times through 0.2 ml droplets of culture medium. Likewise, there was no change when the embryos were treated in the following manner. A group of preliminarily washed 2 cell embryos in a volume of 1–2 μl was placed at the top of a column of 5 ml of culture medium (under 0.5 ml of mineral oil to maintain the pH at 7.2) in a 12 ml conical centrifuge tube. The tube was centrifuged at 2500 rpm (approximately 1000 g) for 10 min, and all but the bottom 0.1 ml of medium was removed. The embryos were recovered from this bottom fraction. Some were saved for assay, and the remainder were transferred in a volume of about 1 μl to a fresh tube and washed in an identical manner a second time. The mean activities of the starting embryos and of those washed one and two times by centrifugation were, respectively, 41.2, 38.4, and 41.1 nmoles/hr/embryo at 37°. Therefore, washing the embryos through large volumes of fluid did did not lead to the loss of any embryonic enzyme activity. These are again at variance with those of Gibson and Masters. They found that washing with either culture fluid or isotonic saline resulted in loss of activity from the ova and in increased activity in the supernatant fluid; 0.25 M sucrose had much less effect.

To further rule out the possibility that there might be enzyme adherent to the surface of the embryo, 2 cell embryos were placed in a solution of 0.5% pronase (Calbiochem) in medium to remove the external zona pellucida. This enzyme is a protease of broad specificity. The embryos were observed to determine when lysis of the zona occurred, and this took between 15 min at 37° and 30 min at 22°. Following lysis of the zona, the surface of the embryos was observed to have become roughened in appearance. Nevertheless, removal of the external coat of the eggs with pronase did not lead to any change in activity: after treatment the mean (± S.E.) activity was 25.9 ± 1.8 nmoles/hr/embryo at 31° as compared to a control value of 22.9 ± 4.1.

Brinster has recently reported that the lactate dehydrogenase activity in oocytes obtained from mouse ovaries is the same as that observed in ovulated ova and early embryos and is about 30 times that found

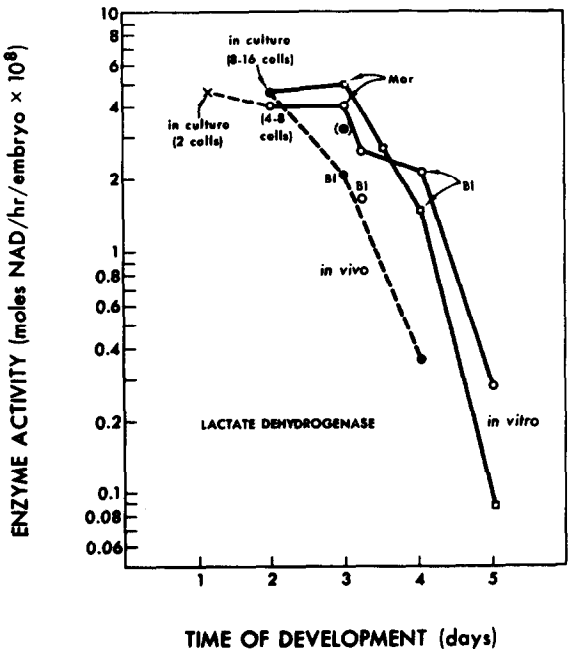

Fig. 1. Lactate dehydrogenase activity of preimplantation mouse embryos cultured in vitro. Embryos were placed in culture at the 2 cell or 8–16 cell stage and enzyme activities measured at various times. (——) cultured embryos; (- - -) embryos developing in vivo. Mor, morula; Bl, blastocyst.

in rabbit and human oocytes [5, 7]. All materials were handled in the same manner. To confirm this observation, follicular oocytes were assayed and were again found to be equal in activity to 2 cell embryos flushed from the oviducts: 22.1 ± 4.3 nmoles/hr/follicular oocyte at 31° as compared to the control value of 22.9 ± 4.1. Thus, the high enzyme activity in the embryos cannot be attributed to adsorption of enzyme from the oviducal fluid since it is already present in the oocyte prior to ovulation. The same has also been shown to be the case for glucose-6-phosphate dehydrogenase (fig. 4 of [1]).

In our initial investigation of lactate dehydrogenase activity, we presented the results of experiments in which the utero-tubal junctions were ligated [1]. The embryos thus forced to develop within the oviduct developed normally, and the decline in lactate dehydrogenase activity occurred on schedule. It was concluded that a change of location of the embryo from

the oviduct to the uterus was not the cause of the decline in enzyme activity. To strengthen this conclusion, two and 8–16 cell embryos have been cultured in vitro, and the results are shown in fig. 1. In both sets of embryos there was a one day lag in the morphological development of the embryos, with blastocyst formation occurring on day 4 rather than on day 3. Nevertheless, lactate dehydrogenase activity did decline, and the decline bore the same relationship to morphological change in vitro as it did in vivo. A similar relationship between biochemical and morphological alterations has also been observed for hypoxanthine–guanine phosphoribosyltransferase, an enzyme which *increases* in activity during the third day of development [2]. Therefore, total maintenance of the embryos either in the presence or in the absence of oviducal fluid does not affect the occurrence of the normal fall in lactate dehydrogenase activity concommitant with blastocyst formation.

Gibson and Masters reported that the predominant lactate dehydrogenase isoenzyme present in washed ova was A4 (or isoenzyme 5), as was that in the washings. However, in the report by Rapola and Koskimies [8], lactate dehydrogenase 1 (or B4) was the only isoenzyme detected by polyacrylamide gel electrophoresis of ova stored at −20°. Likewise, Auerbach and Brinster [9], using cellulose acetate electrophoresis, found 98% of the lactate dehydrogenase was present as the B subunit. Since both Gibson and Masters [3] and we (unpublished) have found that considerable enzyme activity is lost when embryos are frozen for more than a few hours, we repeated the electrophoretic analysis of the lactate dehydrogenase isoenzymes in day 1 (2 cell) embryos. The embryos were washed 4 times in culture medium and *rapidly* frozen and thawed in 10 µl of 0.01 M tris HCl, pH 7.4, containing 0.5% bovine serum albumin. The samples were then *immediately* mixed with 25 µl of a 0.25 M sucrose solution, applied to the gels, and electrophoresed. For comparison, the supernatant of a kidney homogenate containing all 5 lactate dehydrogenase isoenzymes was applied to gels run in parallel. Finally, aliquots of the fluid flushed through the oviducts when the embryos were obtained were also applied to gels. In agreement with the findings of Gibson and Masters, isoenzymes 1 through 5 were found to be present in the oviduct flushings, with greatest activity in bands 4 and 5. However, in complete contrast to their results but in agreement with the other previous reports, nearly all activity in the ova migrated as lactate dehydrogenase 1, with only about 10% present as isoenzyme 2. The embryo pattern was completely different from that of the oviduct flushings and cannot be attributed to artifactual changes resulting from storage.

The evidence presented above can be summarized as follows: 1) the activity of lactate dehydrogenase in early preimplantation embryos of the Swiss mouse is the same as the activity present in follicular oocytes removed from the ovary; 2) lactate dehydrogenase activity in early preimplantation embryos is not affected by thorough washing or by treatment with and removal of the zona pellucida by pronase; 3) the exponential decline in enzyme activity concommitant with blastocyst formation is not influenced by the environment which surrounds the embryos; and 4) while all five lactate dehydrogenase isoenzymes are present in oviduct flushings, virtually all activity in the embryos is in isoenzyme 1.

The reason for the discrepancy between our results and those of Gibson and Masters is not clear. One possibility is that the method of embryo handling employed by these investigators is not optimal and leads to leakage of the enzyme from embryos. Another and much more intriguing explanation would be that there is a strain difference between the Swiss-Webster and Quackenbush strains in the quantity and type of lactate dehydrogenase in the ova. Not having the Quackenbush strain makes it impossible for us to test this possibility directly. If it were true, it would provide an interesting system for the study of gene expression during oogenesis. However, whatever the explanation, we conclude on the basis of the evidence presented in this paper that the lactate dehydrogenase of preimplantation Swiss mouse embryos is present within the embryos and is not adsorbed from the oviducal fluid.

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