Embryonic Enzyme Patterns: Characterization of the Single Lactate Dehydrogenase Isozyme in Preimplanted Mouse Ova

Abstract. *A single lactate dehydrogenase isozyme has been observed in mouse eggs from unfertilized eggs to blastocyst stage. As characterized by electrophoresis, substrate inhibition, and subunit dissociation-recombination experiments, it is identical to mouse lactate dehydrogenase 1.*

It is generally agreed that the five commonly encountered lactate dehydrogenase (LDH) isozymes of higher animals are composed of two different polypeptide subunits, A and B. The five isozymes are formed when the polypeptides A and B combine in all possible combinations of four. The subunit structure of LDH-1 to LDH-5 are thus: A0B4, A1B3, A2B2, A3B1, and A4B0 (*1*). The early embryonic tissues of mammals contain mainly LDH molecules with predominantly, or solely, A subunits (LDH-5 and 4). During development the LDH isozyme patterns of most mammalian tissues change towards a pattern containing more B subunits (*2*). This shift in polypeptide production has been claimed to be an expression of differential gene activation in the cells undergoing differentiation (*2, 3*).

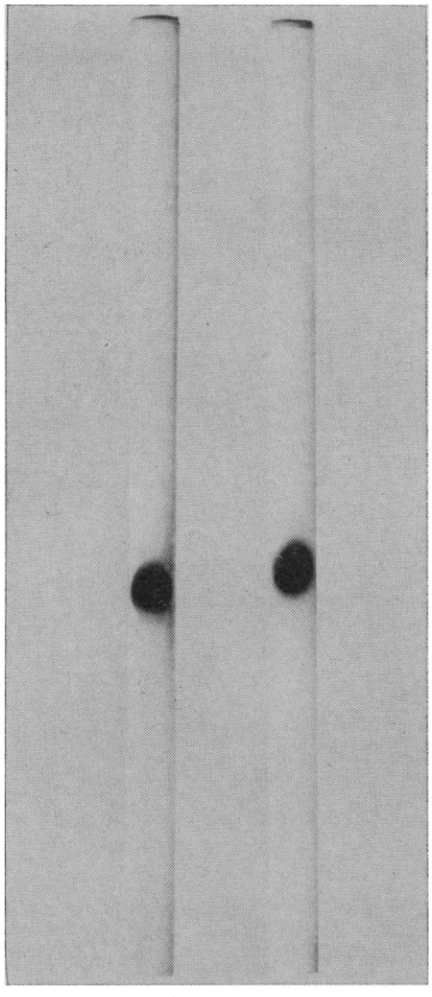

Fig. 1. Electropherograms of mouse egg LDH in small (1.1 by 30 mm) acrylamide columns. One-cell stage eggs in the left-hand column and morula-stage eggs in the right.

Fertilized mouse and rat ova contain high LDH activity (*4*). Auerbach and Brinster have recently reported that the mouse ovum LDH exists in one isozymic form having the electrophoretic mobility of adult mouse LDH-1 (*5*). That exceptional molecular forms of LDH reportedly exist in spermatozoa (*6*) probably reflects the very special metabolic requirements of germ cells. Thus it can be asked whether egg cell LDH is one of the five common isozymes or an exceptional molecular form like that of spermatozoa. The electrophoretic mobility alone is not always a sufficient criterion for characterization of an unknown LDH isozyme (*7*), and additional studies are needed to unravel the nature of the isozyme. Characteristics in relation to reference LDH isozymes can be tested in various ways, catalytic activity in different substrate concentrations being widely used in enzyme chemistry. The tetrameric structure and ability to combine with other isozymes can be tested by dissociation-recombination techniques.

We have also noted that mouse eggs and embryos before implantation have only one LDH isozyme and report here the details of the characterization of this molecular form of LDH which show that this isozyme is identical with adult mouse LDH-1.

Fertilized and unfertilized eggs from superovulated randomly bred Swiss mice were obtained (*8*). The eggs were stored in the minimum amount of 0.1*M* sodium phosphate buffer (*p*H 7.0) and kept frozen (−25°C) until used. Lactate dehydrogenase was liberated from the cells by freezing and thawing. Electrophoresis was carried out in 7.5 percent acrylamide columns (5 mm by 60 mm) (*9*). After electrophoresis the gels were stained for LDH by the tetrazolium method (*2*). When the pattern of LDH isozymes from only a few eggs (about five) was to be analyzed, the electrophoresis was carried out in gels 1.1 to 1.2 mm in diameter and 30 mm long (*10*). Isozymes 1 and 5 from adult mouse tissues were obtained by stepwise addition of ammonium sulphate (40 to 65 percent saturation) to the supernatant of crude tissue homogenate. The precipitate was collected and subjected to electrophoresis, and the bands of LDH-1 and LDH-5 were eluted from the acrylamide gel. Enzyme activity was assayed spectrophotometrically with sodium pyruvate used as substrate (*11*).

Developmental stages from unfertilized eggs to blastocysts showed a single band with high enzyme activity after electrophoresis and demonstration of the LDH activity. The mobility of this molecular form was the same as that of LDH-1 from adult mouse tissues. No trace of other LDH isozymes was detected after electrophoresis of 100 to 200 eggs, combined with prolonged staining (Fig. 1).

We characterized the single isozyme further to determine whether it was

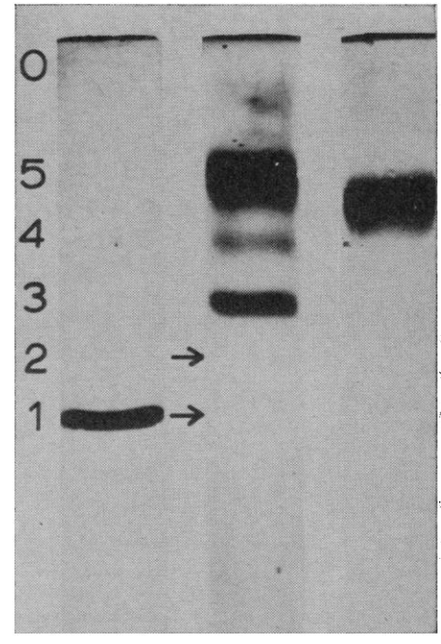

Fig. 2. Electropherograms of LDH from 2- to 4-cell stage eggs (left-hand column) and from adult mouse tissue LDH-5 (right-hand column). Results of dissociation-recombination of the two by freezing and thawing technique are shown in the center column. Arrows indicate faintly stained LDH-1 and LDH-2.

identical to LDH-1 from adult mouse tissues, or was a different molecular form having the mobility of adult LDH-1. A total of 50 to 100 eggs of the same developmental stage were divided into two equal groups, and the LDH activity was assayed in low ($3.25 \times 10^{-4}M$) and in high ($1.6 \times 10^{-2}M$) concentrations of pyruvate. The ratio of LDH activity in the low concentration to that in the high concentration of pyruvate fell in the range of 4.25 to 4.50 in several assays. The same ratio for mouse LDH-5 was 1.9, while that for LDH-1 was 4.50.

The subunit structure of egg LDH was studied by a dissociation-recombination experiment described by Markert (*12*). Egg LDH and LDH-5 were mixed in $0.1M$ sodium phosphate with $0.5M$ NaCl (*p*H 7.0), frozen, and then thawed; and the material was subjected to electrophoresis. Three new bands of LDH activity appeared in the electrophoretic pattern. The recombination under these conditions seemed to favor a combination of two A subunits from the LDH-5 and two subunits from the egg LDH at the expense of the other two heteropolymeric isozymes (Fig. 2).

Our results on the electrophoretic nature of mouse egg LDH agree with those of Auerbach and Brinster (*5*), except that no trace of isozymes other than that with the mobility of LDH-1 was detected. According to our data the mouse egg LDH isozyme is identical with adult mouse LDH-1 in electrophoretic mobility, substrate inhibition, and subunit structure. The exclusive presence of B subunits in the mouse ova before implantation, on the one hand, and the general knowledge that implanted young embryos contain LDH with an overwhelming amount of A subunits, on the other suggest an abrupt change in the activity of the corresponding genes at the time of or shortly after the implantation. Regulation of the differential gene activity is not known, but it has been proposed that tissues undergoing aerobic metabolism contain LDH isozymes with mainly B subunits, whereas tissues capable of anaerobic metabolism exhibit A subunits (*3, 13*). Our results agree with this, since the oxygen uptake of rat and rabbit eggs is significantly higher than that of implanted embryos (*14*). It could be thought that the change from aerobic metabolism of eggs before implantation to the less aerobic metabolism of implanted embryos promotes the initiation of A subunits synthesis, which lasts until implantation is inhibited. Mouse eggs and embryos before implantation are unusual in that they contain only a single LDH isozyme which, to our knowledge, does not exist in any other mammalian cell type, with the possible exception of spermatozoa (*15*).

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16. Supported by grant from the Sigrid Jusélius Stiftelse, the Finnish Medical Research Council and Damon Runyon Memorial Foundation (DRG-938).

5 June 1967

Hydroxyethyl Starch: Extracellular Cryophylactic Agent for Erythrocytes

Abstract. *Studies in vitro indicate that hydroxyethyl starch is an effective extracellular cryoprotective agent for erythrocytes. It is as effective as polyvinylpyrrolidone in cryophylactic ability. It is degraded to glucose units in the circulation, is not retained in tissues, and is inexpensive to produce. Changes in the size and degree of hydroxyethylation of the branched-chain starch may result in a more effective cryoprotective agent.*

A 6-percent solution of hydroxyethyl starch (HES) has been shown to be effective without serious side effects as an expander of plasma in laboratory animals and in man (*1*). Physiologically, it is degraded slowly in the bloodstream, and initial studies suggest a very low degree of antigenicity (*2*). It has been compared to dextran for its plasma-expander properties; however, it is more stable on long-term storage and freezing and thawing than dextran is (*3*), and it is inexpensive to produce (*4*).

In the bloodstream, starch is progressively hydrolyzed by contact with α-amylase. Unaltered starch will not persist in the circulation, but substitution of the starch hydroxyl groups by hydroxyethyl ether groups interferes with the breakdown of the starch by α-amylase.

Other expanders of plasma, such as dextran and polyvinylpyrrolidone (PVP), have been studied for their extracellular cryoprotective activity. Because HES is slowly degraded in the circulation, it seemed reasonable to explore its ability to act as a cryoprotective agent in the freezing of erythrocytes.

Intracellular protective agents, such as glycerol, are now being effectively used for the long-term preservation of erythrocytes. To utilize an intracellular protective agent, it is necessary to remove the material from the erythrocytes prior to administration. An extracellular agent which can be metabolized and could be administered with the cells would have a definite advantage in reducing to a minimum the processing after thawing.

We previously used PVP as an extracellular cryophylactic agent for long-term preservation of erythrocytes by the Linde liquid-nitrogen technique (*5*). Although PVP offers excellent protection to the erythrocyte during the freezing and thawing process,