ON THE LACTATE DEHYDROGENASE OF PREIMPLANTATION MOUSE OVA

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1. Introduction

Lactate dehydrogenase (E.C.1.1.1.27) exists in most mammalian tissues as five molecular forms, whose subunit composition may be represented by the sequence A_4, A_3B, A_2B_2, AB_3, B_4 where A and B are different polypeptides. During embryonic maturation, differential gene function is reflected in the redistribution of activity amongst the multiple forms of this enzyme, and these developmental progressions have been widely utilized in studies of tissue differentiation, and the control of enzyme synthesis in vertebrate systems [1–3].

Among the most salient observations which have been made concerning the ontogeny of this enzyme in recent years, were the reports of extremely high lactate dehydrogenase activities of the B-type which were associated with mammalian ova in the preimplantation stages [4], and the subsequent abrupt decrease in this activity and change of sub-unit type which coincided with uterine attachment [5, 6]. These findings held quite dramatic implications in developmental biochemistry, and provided new insight into the mode of energy utilization of these early embryonic stages. Nevertheless, there remained several puzzling features of the reported phenomenon, – in terms of the relative stability of the different subunit types, and the control of synthesis of this enzyme, for instance.

The present communication describes a re-examination of the early ontogenetic properties of lactate dehydrogenase in the mouse, and reveals a disposition of the enzyme which casts new light on the role of lactate dehydrogenase in early ontogeny.

2. Materials and methods

Mouse embryos were obtained from randomly bred mice of the Quackenbush strain, superovulated by the intraperitoneal injection of 10 I.U. of serum gonadotrophin (Gestyl, Organon), followed 48 hr later by the intraperitoneal injection of 10 I.U. of choronic gonadotrophin (Pregnyl, Organon) [7]. Successive developmental states were removed at appropriate times after ovulation by flushing the reproductive tract with cultute medium (essentially Krebs Ringer bicarbonate, containing 1 mg/ml of crystalline bovine serum albumin, 100 I.U./ml of penicillin and 50 I.U./ml streptomycin, and with 10.15 mM sodium lactate replacing an equivalent molarity of sodium chloride). These preimplantation ova were then thoroughly washed by centrifugation (3 times, 1000 *g*; 10 min) and decantation. After freezing and thawing to release lactate dehydrogenase from the cells, the specific activities and electrophoretic patterns of the enzyme in two and four cell ova were determined and the percentage of B-type contribution calculated by scanning in an integrating densitometer [8, 9, 12].

3. Results and discussion

The results of these analyses were reproducible, and were similar in both the two cell and four cell stages, but differed markedly from previously reported results for mouse preimplantation ova [4–6]. Instead of specific activities several times the level of adult tissues, the values obtained were little different

Table 1
The lactate dehydrogenase activity of preimplantation mouse ova.

Treatment	Specific activity		Percentage B-type	
	Cells	Supernatant	Cells	Supernatant
Washed in culture fluid				
3 times	0.24	0.82	5	25
1 time	0.60	0.38	17	25
Washed in 0.25 M sucrose				
1 time	0.80	0.22	25	25
Washed in isotonic saline				
1 time	0.20	0.80	12	25
Washed in culture fluid				
1 time (deep frozen -20°,				
2 weeks)	0.02	–	85	–
Extracellular, oviducal secretion	–	1.9	–	25

Ova were harvested at the two and four cell stage. Details of washing techniques and enzyme determination are described in the text. Specific activities are expressed as international units per mg protein.

from the much lower values characteristic of early mammalian embryos [9]. Again, instead of an activity distribution of the lactate dehydrogenase isoenzymes which was concentrated in the anodal form (B_4), the activity remaining in the washed ova was predominantly A-type (table 1, fig. 1).

In seeking an explanation of these puzzling discrepancies, the divergences in washing technique for the ova were investigated, (the previously reported procedure involved transfer of ova from one wash fluid to another by manipulation [7], rather than centrifugation in relatively large volumes of fluid as in the present study); and this direct comparison of techniques revealed a possible causation of the differences in enzyme analyses. It was noted that considerable lactate dehydrogenase activity appeared in the supernatant wash fluid after centrifugation of the ova, and that this eznyme contained appreciable B-type activity. Furthermore, loss of enzyme from the ova to the supernatant, and hence the fall in the specific activity of the ova, was proportional to the extent of the washing (table 1).

In order to investigate this behaviour further, some ova were suspended and centrifuged in 0.25 M sucrose, and some in isotonic saline. These procedures were followed by microsopical examination and no alteration in the integrity of the ova was observed. By comparison with the culture fluid, loss of activity from the ova to the supernatant was decreased when sucrose was used, but markedly increased in the case of saline (table 1). Sucrose solutions have, of course, been widely utilized as a stabilizing influence in studies of enzyme localization while saline, in contrast, is known to weaken electrostatic binding of enzymes of cell surfaces. The original wash medium, because of its high content of albumin, may be considered as intermediate in these characteristics between saline and sucrose.

On the basis of these results, then, it is concluded that much of the very high lactate dehydrogenase activity which has been reported to be associated with preimplantation ova, arises by extracellular adsorption. The ova migrate in an environment which is relatively enriched in lactate dehydrogenase, (the oviducal secretion contains high activities of extracellular lactate dehydrogenase) [11]; and in so doing evidently accumulate enzyme on the cell surface with a different isoenzyme composition to that in the cell cytoplasm (table 1, fig. 1).

Additional support for this interpretation comes from previous observations of the adhesive nature of the outer surface of mammalian ova prior to implantation, and the consequent necessity for careful exclusion of possible contamination by activities from

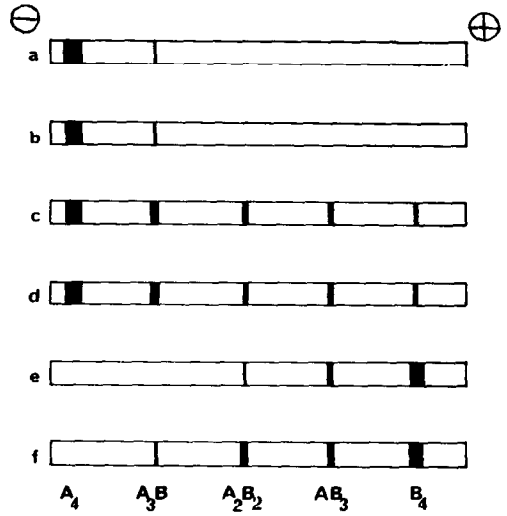

Fig. 1. Polyacrylamide gel zymograms of lactate dehydrogenase
(a) Mouse skeletal muscle
(b) Preimplantation ova, washed three times by centrifugation in culture fluid
(c) Supernatant from b
(d) Extracellular secretion from mouse oviduct
(e) Preimplantation ova, incompletely washed, then deep frozen at -20° for two weeks
(f) Mouse heart.

the endometrial secretion, in studies of embryonic enzymes [10].

It may be noted, also, that the residual enzyme in thoroughly washed mouse ova is predominantly A-type, in the present results, in contradistinction to the previous reports of marked B-type preponderance at this developmental stage [5, 6]. In regard to these differences, a possible explanation is provided by our observations that early embryonic tissues are less stable than most adult sources in regard to their lactate dehydrogenase content, and that the A-type activity is more susceptible to inactivation by prolonged storage at low temperature than B-type activity. Incompletely washed samples of mouse ova, for example, show a marked decrease in A-type activity, and shift to a relatively higher percentage content of B-type contribution with this cold storage (table 1, fig. 1). A reconciliation of the divergent analyses is thus possible on this basis.

In conclusion, then, the data in this communication are not construed as diminishing the established, major importance of lactate dehydrogenase in the development of the early mammalian embryo. They do, however, profoundly influence previous interpretations and present understanding of the biological significance of the enzyme during this period. In relation to the control of enzyme synthesis, for example, the present results point not to a change in the type and extent of lactate dehydrogenase biosynthesis (B to A-type) at implantation which is greater in this short period than the cumulative progressions in the remainder of the maturation process [5, 6], or the occurrence of specific enzyme degrading processes [13], but rather to an alteration in the cell surface and environment of the ova at this time. The latter interpretation would appear to afford good agreement with the established histological correlations of implantation, and with the relatively constant expression of the lactate dehydrogenase genes which has been observed in the early developmental stages of a variety of mammalian species [2, 9].

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