OVIDUCAL LACTATE DEHYDROGENASE

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In recent years, considerable attention has been focused on the rôle of lactate dehydrogenase and its isoenzymes in reproduction and early mammalian development. The study of the developmental progressions of these multiple enzyme forms has greatly advanced our understanding of differential gene control in early ontogeny (Cahn, Kaplan, Levine & Zwilling, 1962; Markert, 1963) and, in addition, it has become evident that the principal reactions of this enzyme (i.e. lactate and pyruvate) are of prime importance as energy sources during the initial stages of cell multiplication following fertilization (Brinster, 1967). Again, the lactate dehydrogenase activities associated with mammalian ova before implantation have been reported to be elevated to a level many times higher than at any other stage of morphogenesis (Brinster, 1965). These findings, with the attendant implications, have established the major significance of this enzyme in early ontogeny.

In the present communication, the lactate dehydrogenase activity in the fluid environment of pre-implantation ova has been studied as an extension of previous investigations into the developmental properties of this enzyme (Hinks & Masters, 1964, 1966; Fieldhouse & Masters, 1966). For this investigation, the reproductive tracts were excised from mature adult females of four mammalian species—rabbits, guinea-pigs, rats and mice. The extracellular secretions within the lumina of the upper oviducts were collected, and the enzyme content of the supernatant fraction examined after centrifugation at 100,000 *g* for 30 min at 4° C. Lactate dehydrogenase activities were assayed, and these fluids were also subjected to zone electrophoresis on polyacrylamide gels and the sub-unit composition of the enzymes was calculated. Substrate activity ratios against pyruvate and oxobutyrate were also determined. The methods involved have been described in detail elsewhere (Hinks & Masters, 1964).

The results of these analyses are summarized in Table 1. They show the high levels of lactate dehydrogenase activity which are found in this fluid, and the major contribution of A sub-unit expression in these enzymes.

In regard to the first of these findings, it is relevant to recall that levels of extracellular lactate dehydrogenase are generally two or three orders of magnitude below the specific activities characteristic of the major tissues and organs (Wilkinson, 1962). In contrast to the usual situation, however, the activity of lactate dehydrogenase in oviduct fluid is of the same magnitude as in some of the richest mammalian tissue sources, such as the heart. This high concen-

tration and extrinsic localization of enzyme activity in the oviducal secretion is noteworthy, in that we know of no other comparable accumulation outside the tissue cells of normal mammals of any other enzyme which is so heavily implicated in a major pathway of intracellular metabolism. Furthermore, although other enzymes are detectable in the fluid of the Fallopian tube, their activity is appreciably lower than that of lactate dehydrogenase. Since the pre-implantation ova are immersed in this selective environment, and dependent upon it for growth and development, the present data would appear to supply further evidence for the involvement of lactate dehydrogenase in early ontogeny. This extracellular activity should be included in any consideration of overall cellular metabolism in the initial stages of growth.

The sub-unit composition of lactate dehydrogenase activity present in the oviduct is also of importance in assessing the rôle of this enzyme in early morphogenesis. Lactate dehydrogenase (EC 1.1.1.27) exists in most mammalian tissues as five molecular forms, each of which is a tetramer; and the multiplicity

TABLE 1

THE LACTATE DEHYDROGENASE ACTIVITY OF THE EXTRACELLULAR FLUID IN MAMMALIAN OVIDUCTS

Species	Specific activity	Substrate activity ratios	Oviducal lactate dehydrogenase isoenzyme contribution				
			A ₄	A ₃ B	A ₂ B ₂	AB ₃	B ₄
Mouse	1.9	0.20	43	27	20	6	4
Rat	1.6	0.30	51	26	16	6	1
Guinea-pig	0.7	0.45	24	35	21	17	3
Rabbit	0.9	0.65	6	10	24	38	22

Specific activities are expressed as international units of activity per mg of protein. The methods of assessment of isoenzyme composition are described in the text. The figures quoted are the means of three analyses for each species. For the purposes of comparison, the lactate dehydrogenase in mouse heart was calculated as 0.8 i.u./mg protein, and 85% B-type.

of the enzyme may be accounted for by the presence of two types of sub-unit (A and B), each of which is under the control of separate genetic loci. Hybridization between the different polypeptides allows for a sequence of heteromorphs whose sub-unit composition may be represented as A₄, A₃B, A₂B₂, AB₃, B₄ (Markert, 1963). Previously, the preferential advantages of B-type lactate dehydrogenase activity to the pre-implantation ova have been stressed, on the basis of the comparatively aerobic environment before uterine attachment and the efficient utilization of lactate in the Fallopian tube (Auerbach & Brinster, 1967; Rapola & Koskimies, 1967). From the present results, the major contribution of A-type activity in this extrinsic location would appear to necessitate a re-assessment of these interpretations.

In summary, the extracellular fluid within the mammalian oviduct has been established as a potent source of lactate dehydrogenase activity. This localization and the sub-unit type of the enzyme, contrast with previous emphasis and would appear to provide a new aspect to the study of mammalian reproduction.

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