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Anomalous multiplicities of lactate dehydrogenase

In most vertebrate tissues, lactate dehydrogenase (EC 1.1.1.27) exists as 5 separate molecular forms which may be differentiated by electrophoretic, immunological and kinetic criteria[1–3]. Furthermore, the individual isoenzymes have been shown to be tetramers formed by the combination of two types of polypeptide subunit, each of which is under the control of separate genetic loci[4–6]. These facts, then, account for the majority of the observed properties of lactate dehydrogenase in vertebrate tissues, and as a consequence, have gained wide acceptance as a basis for the interpretation of the developmental progressions and structural characteristics of this enzyme.

On occasion, however, lactate dehydrogenase distributions are observed which are not readily reconcilable with this scheme. Studies of the ontogeny of the enzyme in this department, for example, have uncovered two separate examples of anomalous macroheterogeneity*. In view of the widespread use of this enzyme in tissue differentiation studies[7,8], and the importance of lactate dehydrogenase as a model for other isoenzyme systems[11], these atypical distributions have been reported in this communication, along with some brief comments on their nature and significance.

The detailed methodology used in these investigations has been described in full previously[8,10]. Homogenates of relevant tissues were subjected to zone electrophoresis on horizontal starch gels, and the multiple forms of lactate dehydrogenase demonstrated by a tetrazolium dye technique. The assignment of the band numbers was effected by direct comparison of the mobilities with the lactate dehydrogenase forms in adult heart and muscle.

As may be observed in Fig. 1a, guinea-pig embryo provides one example of a mammalian tissue which displays an atypical distribution of lactate dehydrogenase

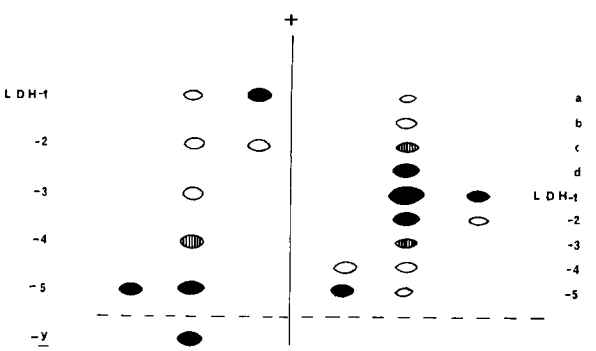

Fig. 1. a. Lactate dehydrogenase zymograms of guinea-pig tissues. Adult skeletal muscle (left), embryo 9 days after fertilization (centre), and adult heart (right). b. Lactate dehydrogenase zymograms of duck tissues. Adult skeletal muscle (left), liver 15 days after fertilization (centre) and adult heart (right).

Abbreviation: LDH, lactate dehydrogenase.

* *i.e.* dispositions affecting the distribution of activity between major bands, rather than sub-banding or microheterogeneity.

multiple forms. At the stage of development immediately prior to the initiation of functional differentiation, six regions of formazan disposition are resolved under the experimental conditions utilized. In addition to the five anodic bands which are distinguishable in more mature tissues and which correspond to the AB set of isoenzymes (*i.e.* LDH 1–5), a further strongly staining region of activity is evident on the cathodic side of the origin (Band Y). Although the full significance of this latter activity and its inter-relationships with bands LDH 1–5 are not yet completely elucidated, some of the possibilities may be eliminated. Since similar protein loadings (10 μg) from more mature tissues showed no indication of this type of activity (Band Y), and since 5-fold dilution of the embryonic homogenates did not alter the relative intensity of the bands, this atypical distribution does not seem attributable to an overloading artifact. Again, Band Y did not stain in the absence of substrate, nor when ethanol was substituted for lactate in the staining system, so non-specific dehydrogenase activity would seem to be contraindicated. Furthermore, no preferential staining with α-hydroxy butyrate, or α-hydroxy caproate was evinced, and this would appear to exclude LDH-C as a contributory factor[9]. Conformational isomerization, the banding of small molecules to an lactate dehydrogenase tetramer, and a distinctive sub-unit type of the enzyme remain to be considered as possible interpretations.

A further anomalous pattern of lactate dehydrogenase isoenzymes was observed during an investigation of the characteristics of this enzyme in avian species (Fig. 1b). A wide range of tissues taken at various stages of development in the duck (*Cairina moschata*) exhibited bands which were additional to the AB set (LDH 1–5) with a maximum of nine separate bands being resolved. Amongst other relevant results, the belated discovery of additional bands of this type raises difficulties in nomenclature[13]. In this case the bands have been designated a, b, c, d (Fig. 1b) in order to preserve a consistency of numbering in the AB set, and also to distinguish the atypical occurrence of the additional heteromorphs. The substrate specificity and dilution characteristics of these bands (see tests discussed for cavian embryo) again indicate that they represent factual lactate dehydrogenase activity, and the substrate specificity and mobilities of these extra bands do not correlate with the properties of LDH-C hybrids in this species. Furthermore, similar patterns to those in the duck (though less intense) have been observed by us in a number of other avian species, whereas mammalian tissues under identical conditions gave no evidence of additional anodic heterogeneity. This anomalous behaviour, then, may be considered of especial interest in view of the significant role played by avian species in establishing interpretations of the ontogeny, functional significance and structural inter-relationships of this enzyme[4,6].

Although other workers have reported that atypical lactate dehydrogenase bands occur with another bird, the penguin[12], and have suggested that these forms represent permutations or modified versions of the normal isoenzymes, it is as well to note that the tissues of the duck present patterns which are significantly different. Instead of an intercalated distribution as in the penguin, the atypical bands in the duck are extralimital to the AB set, and decrease in intensity sequentially with distance from the origin. This, and the developmental behaviour seem more consistent with the expression of an additional gene in these avian tissues.

In summary, then, these findings illustrate that early developmental tissues may contain more than five electrophoretic forms of lactate dehydrogenase activity. In view of this anomalous multiplicity, the assignment of relative mobilities and the

interpretation of kinetic data for lactate dehydrogenase in these sources may be seen to require especial care and adequate corroboratory evidence. The normal adult situation when the ratio of sub-unit type (A:B) of lactate dehydrogenase correlates directly with many of the kinetic and physicochemical characteristics may not necessarily pertain in the early ontogeny of the enzyme.

Further studies aimed at the elucidation of these problems are in progress in this department at present.

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REFERENCES

1 C. L. MARKERT AND F. MOLLER, *Proc. Natl. Acad. Sci. U.S.*, 45 (1959) 753.
2 P. G. W. PLAGEMAN, K. F. GREGORY AND F. WROBLEWSKI, *Biochem. Z.*, 334 (1961) 37.
3 I. H. FINE, N. O. KAPLAN AND D. KUFTINEC, *Biochemistry*, 2 (1963) 116.
4 D. T. LINDSAY, *J. Exptl. Zool.*, 152 (1963) 75.
5 C. L. MARKERT AND E. APELLA, *Ann. N.Y. Acad. Sci.*, 94 (1961) 678.
6 R. D. CAHN, N. O. KAPLAN, L. LEVINE AND E. ZWILLING, *Science*, 136 (1962) 962.
7 C. L. MARKERT AND H. URSPRUNG, *Develop. Biol.*, 5 (1962) 363.
8 B. FIELDHOUSE AND C. J. MASTERS, *Biochim. Biophys. Acta*, 118 (1966) 538.
9 W. H. ZINKHAM, A. BLANCO AND L. KUPCHYK, *Science*, 144 (1964) 1353.
10 M. HINKS AND C. J. MASTERS, *Biochemistry*, 3 (1964) 1789.
11 C. J. MASTERS, *Biochem. Biophys. Res. Commun.*, 28 (1967) 978.
12 C. L. MARKERT AND W. J. L. STADIN, *Nature*, 210 (1966) 948.
13 E. C. WEBB, *Experientia*, 20 (1954) 592.

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