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Four Types of Genetic Variants of LDH Found in India

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Abstract. The four types of genetic variants of lactate dehydrogenase (LDH) found so far in the Indian populations surveyed by starch-gel electrophoresis of haemolysates are described, pointing out their characteristic features. Two of these variant types, namely, 'Calcutta-1' and 'Madras-1', were reported earlier. The other two discovered subsequently and named trivially as 'Calcutta-2' and 'Delhi-1' are now reported for the first time. A comparison between the four LDH variants found in India and those by others outside India has also been drawn.

Key Words
LDH variants
Indian population study

Introduction

Investigations into the genetic variants of several enzymes from erythrocytes and placenta by means of starch-gel electrophoresis were started at our institute in 1968 in collaboration with Dr. R. L. Kirk (Canberra). Several Indian populations have since been screened for a number of enzyme systems, including lactate dehydrogenase (LDH, EC 1.1.1.27), and some of the results have been published [Das *et al.*, 1970a, b, c]. Ananthakrishnan *et al.* [1970] and Blake *et al.* [1970] have reported on their findings among certain Indian populations.

These investigations and our recent studies have revealed four different genetic variants of LDH in India; the two designated as (1) 'Calcutta-1' and (2) 'Madras-1' were reported earlier [Das *et al.*, 1970a, b]; the other two found recently and designated (3) 'Calcutta-2' and (4) 'Delhi-1' are reported here. They will henceforth be referred to as 'LDH Cal-1', 'LDH Mad-1', 'LDH Cal-2' and 'LDH Del-1', respectively.

'LDH Cal-1' was first detected in Calcutta and was investigated in a pedigree in which a man of the Mahishya caste, one of his three sons and the latter's son had this enzyme variant in a heterozygous condition. Further studies in different parts of India have proved that 'LDH Cal-1' is distributed fairly widely among the peoples of India of various linguistic groups, with frequencies ranging from below 1 to little above 4% (probably more frequent in the so-called lower castes than in the upper castes [DAS *et al.*, 1972]).

'LDH Mad-1' has been noticed only in two Madras samples from the Chettiar and the Naidu castes (Tamil-speaking). No pedigree study was done with respect to this variant.

'LDH Cal-2' has been found in a Bengali Muslim family of West Bengal in the father and one of his sons. Another example of this variant has come to our notice in the Muslim.

'LDH Del-1' has so far been encountered in a solitary blood sample from a man of the Ahir caste of Delhi, where about 500 persons were sampled from five different castes. No pedigree has yet been studied.

The normal and Common LDH

The population of the world screened to date for LDH variants have been found to possess predominantly the normal type of this enzyme [VESELL, 1965; BECKMAN, 1966; MOURANT *et al.*, 1968; KIRK *et al.*, 1969; BLAKE *et al.*, 1969; etc.]. The variants, if any, form altogether a very small fraction of the population; let us say, from below to slightly above 1%. Higher rates of incidence have been found in India [DAS *et al.*, 1970a; ANANTHAKRISHNAN *et al.*, 1970], where even as high as 4% of 'LDH Cal-1' has been recorded for the Nadar caste.

The normal LDH has five isozymes, which are nothing but five possible combinations of two independently formed subunits (polypeptide chains), A and B, made into tetrameric LDH molecules: B_4, B_3A, B_2A_2, BA_3, and A_4 [APELLA and MARKERT, 1961; MARKERT, 1962, 1963; BECKMAN, 1966]. In the zymogram of normal LDH (i.e., properly processed and visualised starch-gel electrophoretic pattern), five widely separated bands may appear, corresponding to the above-mentioned five molecular combinations of LDH.

LDH from erythrocytes or from the heart, however, shows only three isozyme bands clearly (fig. 1). Enumerating from the anodal end, they respectively correspond to the isozymes, LDH-1 (B_4), LDH-2 (B_3A) and LDH-3 (B_2A_2). The zymograms of LDH from liver and kidney, on the other hand, prominently exhibit the four bands corresponding to LDH-2, LDH-3, LDH-4 (BA_3) and LDH-5 (A_4) with gradually increasing intensities. LDH-1 is extremely faint or invisible. The five isozyme bands for normal LDH are always single bands. The LDH-5 band appears on the cathodal side of the line of application, unlike the rest, which come on the anodal side [YAKULIS *et al.*, 1962].

Mutations in the A or the B Subunit

The formation of the subunits A and B is under independent gene control. Two loci are involved – one for each subunit [SHAW and BARTO, 1963; NANCE *et al.*, 1963; DAVIDSON *et al.*, 1965; BECKMAN, 1966]. A mutation causing a variation in the A subunit and a consequent change in the mobility of the A subunit and a consequent change in the mobility of the constituted LDH molecules (most likely in proportion with the number of mutant subunits contained in them) would result in a splitting of the isozyme bands into two or more components in the heterozygotes. Thus, the bands of isozymes LDH-2,-3,-4, and -5 each resolve respectively into 2, 3, 4 and 5 components, LDH-1 obviously remaining single. In a parallel fashion, a mutation in the B subunit would cause a resolution of the bands of LDH-1, -2, -3 and 4- respectively into 5, 4, 3 and 2 components, while LDH-5 would remain single.

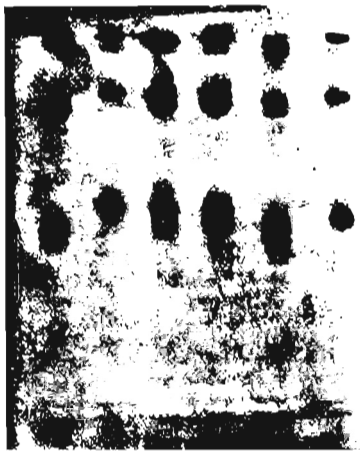

Fig. 1. Photograph of starch-gel electrophoretic patterns of LDH variants in haemolysates of man (phosphate-citric acid buffer, pH 7.0). 1, 6: normal; 2: homozygous 'Cal-1'; 3: heterozygous 'Cal-2'; 4: heterozygous 'Cal-1'; 5: 'Del-1'.

The alteration in the mobility (anodal) of the LDH molecules mentioned above may, however, be either an increase or a decrease from the the normal. In 'LDH Cal-1' and 'LDH Cal-2', we have two distinct A-subunit variations, each producing its own characteristic 'faster-than-normal' LDH variant, 'LDH Cal-2' being even faster than 'LDH Cal-1' in the appropriate sense (fig. 1). 'LDH Mem-1' and 'LDH Mem-2' [KRAUS and NEELY, 1964] are both examples of 'faster' A-subunit variations, the latter being even 'faster' than the former.

'LDH Mem-4' of KRAUS and NEELY [1964] is an instance of an A-subunit variation which has resulted in a 'slower-than-normal' LDH variant found in a 'white'.

The cases of B-subunit variations noticed to date are all of the 'slower-than-normal' variety. They are: 'LDH Mem-3' [KRAUS and NEELY, 1964], 'LDH Mad-1' [DAS *et al.*, 1970a], and 'LDH Del-1' of the present study (fig. 1, 2).

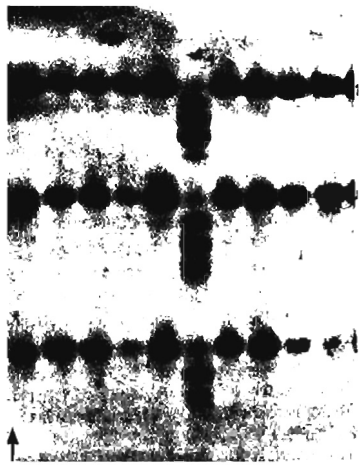

Fig. 2. Photograph of starch-gel electrophoretic patterns of LDH variants in haemolysates (man). The 6th from right: 'LDH Del-1' heterozygote. Rest normal.

Assuming that the mutant subunits for A or B behave chemically as their normal counterparts in forming the tetrameric LDH molecules, it is expected that the relative intensities of the components of an isozyme would fit closely with the ratios, 1:1, 1:2:1, 1:3:3:1 and 1:4:6:4:1, according to whether there are two, three four or five components of that isozyme [DAVIDSON *et al.*, 1965].

In the zymograms which we obtained from our 'LDH Del-1' sample (fig. 2), we had a clear confirmation of the above-expected intensity relationship among the components of the isozymes, as the patterns were very well reproduced in photographs that showed clean spaces in between the components, which were also mutually well separated. Copies of the photographs were also referred to Prof. H. HARRIS, FRS (Galton Laboratory), and Dr. R. L. KIRK (Canberra), both of whom confirmed our views.

Homozygous LDH Variants

Occurrence of homozygous variants of LDH must be extremely rare. VESELL [1965] reported one case of homozygosity and another has been reported by ANANTHAKRISHNAN *et al.* [1970]. The latter seemed to be homozygous 'LDH Cal-1', showing, as expected, four single bands corresponding to LDH-1, LDH-2, LDH-3, and a very faint LDH-4. All the bands, except that for LDH-1 which is due to B_4, are anodally displaced for reasons already explained.

Summary of the Indian LDH Variants

(1) 'LDH Cal-1', first discovered in Calcutta, traced through three generations in a pedigree, is found widely distributed among Indian peoples, the frequency varying from below 1 to even above 4%. *It is a 'faster A-subunit' variant.*

(2) 'LDH Cal-2', found and traced through two generations of a Muslim family of West Bengal, has recently been encountered in another Muslim. *It is also a 'faster A-subunit' variant*, but the mutant A subunit of 'LDH Cal-2' is even faster than that of 'LDH Cal-1'.

(3) 'LDH Mad-1' has been detected in Madras in two individuals, but in no other place so far. *It is a 'slower B-subunit' variant.*

(4) 'LDH Del-1' has been detected so far only in a man of Ahir caste of Delhi. It is also a *'slower B-subunit' variant*, but the mutant B subunit of 'LDH Del-1' is much slower than that of 'LDH Mad-1'.

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