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GENETIC VARIANTS OF LACTATE DEHYDROGENASE IN INDIA

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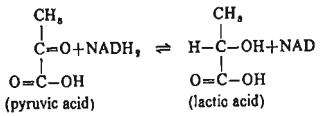
ABSTRACT : The paper reviews the work carried out on the lactate dehydrogenase variants among Indian populations. Of the four different genetic variants of LDH found in India, only the Calcutta-1 variant appears to be polymorphic in the populations. Other variants are of sporadic occurrence. The Calcutta-1 variant shows higher frequency among the lower caste populations both in West Bengal as well as in Tamil Nadu. The Brahmans of both the areas, on the other hand, show lower percentage of the trait.

INTRODUCTION

Our knowledge about the distribution of the genetically determined variants of the various enzymes from erythrocytes or from other tissues of man in India is still very meagre. The enzyme glucose-6-phosphate dehydrogenase (G-6-PD) has, however, received fairly good attention (Chatterjea, 1966 & 1969). Recently, several enzyme systems are being studied in Indian peoples from erythrocyte and placenta under the leadership of Dr. R.L.Kirk (Canberra). Some of the results have already been published (Das et al., 1970a, 1970b & 1970c ; 1972a & 1972b ; Anathakrishnan et al., 1970 ; Blake et al., 1970 ; Undevia et al., 1972).

In this paper, we are reviewing the work carried out on lactate dehydrogenase (E.C.1.1.1.27 ; LDH) among Indian populations. This enzyme is present in the soluble phase of the cells and catalyses the reversible reduction of pyruvic acid to lactic acid with $NADH_2$ as the reducing agent.



The main source of energy in tissues is the oxidation-reduction reactions catalysed by the Oxydases and Dehydrogenases, the dehydrogenase-mediated ones being the most common. In the presence of an excess of lactic acid, the reaction proceeds from the right to the left, liberating $NADH_2$. (NAD = nicotinamide adenine dinucleotide, a cofactor),

LDH is ubiquitous in all mammalian tissues and has been studied in relation to conditions of health, disease, development (ontogenic variation), and organ or tissue variations (Kar and Pearson, 1963; Yakulis et al., 1962; Van Der Helm et al., 1962; Wieme and Van Maercke, 1961; Vesell et al., 1962; Wroblewski and Gregory, 1961; Markert and Moller, 1959; and others).

THE MOLECULE OF LACTATE DEHYDROGENASE

The enzyme LDH is a protein which has a molecular weight of 135,000. A molecule of LDH can be dissociated with 5M guanidine-HCl into four subunits or polypeptide chains of equal size. These subunits or polypeptide chains are found to exist in two different electrophoretic varieties which has been designated as A-subunit and B-subunit (Apella and Markert, 1961: Markert, 1962 & 1963). The two subunits are also immunologically distinct (Cahn et al., 1962).

The subunits A and B freely combine to form tetrameric LDH molecules of the compositions, A_4, A_3B, A_2B_2, AB_3, and B_4 which give rise to five isozymes of this enzyme.

A nearly similar process works in the synthesis of the protein moiety of human haemoglobin in the adults. Here dimers of the two independently formed polypeptide chains, α and β, firstly formed, and the dimers then combine together to form the tetramer, $\alpha_2\beta_2$.

Markert (1963) has been able to dissociate the molecules of LDH-1 (B_4) and LDH-5 (A_4) into respective pure subunits by freezing them in 1M NaCl. After thawing and mixing up the two kinds of submits A and B, the latter combined *at random*, without any preference, into tetrameric molecules to yield all the five isozymes in the theoretically expected ratio of 1 : 4 :6 :4 :1 very nearly.

The question that naturally arises now is that whether the A and the B subunits differ in catalytic activities or not. Sufficient evidences have been produced, which establish that the two subunits are significantly different in their amino-acid contents and structure and also in their function and activity characteristics (Markert and Urspring, 1962; Cahn et al., 1962; Fondy and Kaplan, 1964; Kaplan, 1964). The studies included several species, fish, amphibian, bird, mammal, etc., including man. Fingerprint techniques were employed to reveal the chemical constituents (Fondy et al., 1964) and standard biochemical methods indicated their relative activity characteristics (optimal substrate concentration, Km values, analogue ratios, turnover numbers).

METHODS

Electrophoresis on Agar gel (Ion Agar No.2), or Acrylamide gel, or better on Hydrolysed Starch gel (Connaught Laboratories, Canada) under suitable experimental conditions and processing for visualization can show up the five isozyme bands of LDH, normal or variant (Lawrence, 1964). We have been following the techniques of Blake et al. (1969), using hydrolysed starch gel with excellent results.

THE ZYMOGRAM OF LDH

The visualised electrophoretic patterns obtained by the method stated above are known as zymograms. The five molecular forms of LDH (normal), also called isozymes, produce five widely separated coloured bands. It has been the common practice with the geneticists to name the isozymes as LDH-1, LDH-2, LDH-3, and LDH-4 and LDH-5 counting them

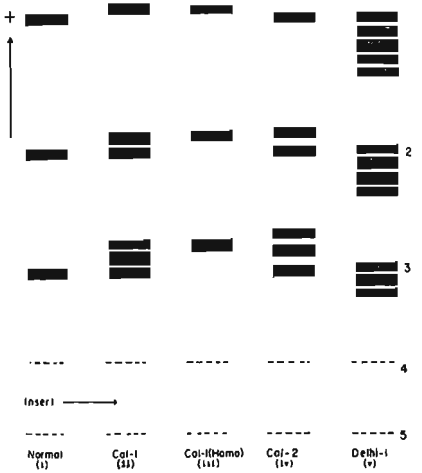


Diagram of Starch gel electrophoretic patterns of LDH variants in haemolysates (man).

(i) Normal LDH, (ii) Heterozygous LDH Cal-1, (iii) Homozygous LDH Cal-1, (iv) Heterozygous LDH Cal-2 and (v) Heterozygous LDH Delhi-1.

from the anodal end of the gel toward the cathodal. The isozyme band for LDH-5 appears on the cathodal side of the line of application. LDH-1 is, therefore, the fasted isozyme. It is B_4. And the isozyme LDH-5 is A_4. Some authors (Lawrence, 1964; Yakulis et al., 1962) have, however, termed the anodally fastest band of B_4 to represent LDH-5 and the cathodally moved band of A_4 to represent LDH-1, a practice quite reverse to the one cited above.

TISSUE VARIATION IN ISOZYME PROPORTION

The LDH zymograms from different tissues or organs show marked deviations in relative intensities from the ideal ratio discussed above owing to variations in the proportion of the A and the B chains produced in different organs. A genetic explanation has been given in a later section.

When the erythrocytes or rather the haemolysates are used as source of LDH, only the isozyme bands for LDH-1, LDH-2 and LDH-3 appear in the zymograms. LDH-4 is occasionally visible, but LDH-5 never show up. Quite contrasting results are noticed in the zymograms produced by LDH from the liver or the kidney. Here, LDH-5 comes out as the most prominent isozyme band with a gradual decline of intensity as one moves towards the anode end of the gel, LDH-1 being just visible, if at all. Heart again, gives a picture similar to that of erythrocytes (Yakulis et al., 1962).

Previously, it was believed that the extracts of muscles (skeletal) in man were most prominent in LDH-5 and progressively weaker in the other isozymes almost like liver or kidney, (Cahn et al., 1962). But this notions has since altered. Kaplan and Cahn (1962), and Kar and Pearson (1963) have produced evidences showing extreme variation among the various skeletal muscles. Some muscles were like the heart and erythrocytes, while there were others resembling liver and kidney in LDH isozyme patterns. Again, a few were intermediate between the above two extreme groups.

ONTOGENIC VARIATION IN ISOZYME PROPORTION

Perhaps a few words on ontogenic variation in isozyme proportion in man are necessary to clear up our understanding. Markert and Urspring (1962), and Kar and Pearson (1963) have important contributions to this aspect of variation. It is reported that the LDH isozyme patterns for various muscles and even liver of the human fetus show a great concentration of enzymic activity in LDH-1 isozyme and also in LDH-2 (in adult liver the highest concentration is in LDH-5).

It implies that in man, the B-subunits predominate in the early fetal life, and that the A-subunits make their appearance in sizable proportions only later in development. Further confirmatory studies on this question of developmental genetics and fetal physiology should prove promising and rewarding. As the development progresses, the various tissues and organs mature, and their characteristic LDH isozyme proportions are assumed as in adults.

OUR OBSERVATIONS IN PLACENTAL TISSUES

Studies were conducted on LDH from placental extracts, carefully avoiding the maternal tissues of placentae. Most interestingly, it is found that the bands for LDH-5 and LDH-4 appear prominently along with the other bands. The placenta, it seems, in addition to its well-known functions in fetal maintenance, growth and development in various ways also serves as the most important seat or perhaps the only seat of LDH-5 and LDH-4 (i.e. A-subunit activity) catalytic action.

There are some evidences which indicate that LDH-5 is less sensitive to pyruvic acid concentration changes than LDH-1. So, LDH-5 is found in tissues where glycolysis is the chief source of energy (skeletal muscles), and contrarily, LDH-1 is present in the tissues which carry on mostly aerobic metabolism (heart) as pointed out by Kar and Pearson (1963). An understandable genetic interpretation of the phenomena may be found in the work of Kaplan (1964).

Accepting the above views, an important question on fetal metabolism naturally arises; whether the fetal body tissues, rich in LDH-1 or B-subunits, are primarily sustained by the aerobic type of metabolism, and that if glycolysis takes place only or mostly in placenta. Further critical studies should elucidate the problem.

THE GENETIC CONTROL OF LACTATE DEHYDROGENASE

The production of the A and the B subunits is under separate gene control. Two loci are involved (Shaw and Barlo, 1963). All observations accumulated for man conform with this hypothesis (Kaplan, 1964; Beckman, 1966).

A mutation can occur at either of the loci, giving rise to a variation in the corresponding A or the B subunit. Simultaneous variations at both the loci must be too rare a phenomenon to encounter one in practice. The mutant subunits may be either slower or faster than the respective normal chains, resulting in slower or faster LDH isozyme variants. Moreover, each isozyme constituted of the normal and mutant

subunits will resolve into an appropriate number, two to five, of components. The isozyme containing no mutant subunit remains single as in normal LDH. Thus, for a A-chain mutation LDH-1 (B_4) gives a single band like the normal LDH, but as stated above, LDH-2 (B_3A), LDH-3 (B_2A_2), LDH-4 (BA_3) and LDH-5 (A_4) give respectively two, three, four and five components instead of the normal single bands.

The normal allele in each case is co-dominant with the mutant alleles. This explains the above splitting of the bands.

THE GENETIC VARIANTS OF LDH IN MAN

A number of genetic variants of LDH have been detected in man by zymogram studies. Kraus and Neely (1964) recorded four different variants, named as Memphis-1, -2, -3 and -4. The first and the second ones were faster A-subunit variants, the third a slower B-subunit variant and the fourth one was a slower A-subunit variant.

Many other workers also have reported genetic variants in various populations in different parts of the world, but their identification with a known type has not always been attempted. We may cite for instance the findings of Davidson et al. (1965). They found two types of variants: one was a slower A-subunit variant and the other was a faster A-subunit variant. But it is unknown if they were identical with Memphis-4 (former) and Memphis-1 or -2 (latter). The slower B-subunit variant detected by Boyer et al. (1963) in a Nigerian also remains as an isolated observation.

In India, we have so far come across four different genetic variants (Das et al., 1970a & 1972b). They have been trivially named as Calcutta-1, Calcutta-2, Madras-1 and Delhi-1. The first and the second ones are both faster A-subunit variants, the mutant subunit in Calcutta-2 being even faster than that in Calcutta-1. The third and the fourth ones are both slower B-subunit variants though quite distinct, the mutant subunit in Delhi-1 being still slower than that in Madras-1. It has been possible for us to compare these four variants mutually in our laboratory. Blake (1971) claimed that he saw a variant in the father and his son of a European family, which was exactly like our Calcutta-2 variant. But he did not suggest any name for it.

We also think on the basis of zymograms that the Delhi-1 variant is quite distinct from the slower B-subunit variant of Boyer et al. (1963), the degree of separation between components of LDH-5, -4, -3 and -2, being definitely much larger than in those of the variant found by Boyer et al. (1963).

TABLE 1

Incidence rates of LDH Calcutta-1 variant

(The figures within parentheses in column 2 give the numbers of the Calcutta-1 variant in the number tested)

Population (1)	*No. tested* (2)	*Cal-1 % ± S.E.* (3)	*References* (4)
WEST BENGAL			
Mixed Bengali Hindus	524 (8)	1.53 ± 0.53	Das et al. (1970a)
Upper caste „ „	493 (2)	0.41 ± 0.26	Das et al. (1972a)
Lower caste „ „	937 (17)	1.81 ± 0.44	-do-
Mixed Indian (Non-Hindu)	90 (2)	2.22 ± 1.60	Das et al. (1970a)
TAMIL NADU			
Brahmin	323 (3)	0.93 ± 0.53	Ananthakrishnan et al. (1970)
Naicker	168 (5)	2.98 ± 1.28	-do-
Nadar	145 (5+1*)	4.14 ± 1.65	-do-
Reddiar	148 (0)	—	-do-
Other mixed Hindus	365 (11)	3.56 ± 0.97	-do-
Non-Hindus	22 (0)	—	-do-
BOMBAY			
Marathi speaking	504 (9)	1.79 ± 0.19	-do-
Gujarati speaking	501 (5)	1.00 ± 0.14	-do-
PUNJAB			
Khetri (Up. caste Hindu)	90 (0)	—	Mukherjee et al. (1971)
Arora („ , „)	43 (0)	—	-do-
Parsis (Bombay)	418 (15)	3.59 ± 0.91	Undevia et al. (1972)
Zorostr. Iranis (")	48 (0)	—	-do-

*Most probably a case of homozygous Calcutta-1 variant

The haemolysate sample which showed the Delhi-1 variant gave unusually clear zymograms with the background very clear. This enabled us to take ideally perfect photographs thereof. As a result, the intensity relationship between the components of each isozyme could be estimated somewhat reliably by inspection. This relationship agreed with the expected ratios on the basis of Markert's theory (1963). These ratios were for LDH-1, -2, -3 and -4 as 1 : 4 : 6 : 4 : 1, 1 : 3 : 3 : 1, 1 : 2 : 1 and 1 : 1 respectively, this is obviously lending a support to Markert.

Copies of the photograph of the zymogram were referred to Dr. R.L.Kirk and to Professor H. Harris, F.R.S. who agreed with our interpretation Das et al., 1972b.

DISTRIBUTION OF LDH VARIANTS IN INDIA

Not much has been done to study the distribution of these enzyme variants in Indian populations. Survey of many other groups all over India, tribal and non-tribal, is urgently necessary. The available information is presented in table 1.

It may be pointed out that of the four genetic variants found in India, only the Calcutta-1 variant appears to be polymorphic in the populations. The rest seem, till today, to have only sporadic occurrence. The table 1, therefore, gives only the distribution of the Calcutta-1 type and not of the other three.

We have already evidences sufficiently strong to suppose that the so-called lower caste groups (in West Bengal and Tamilnadu at least) possess the Calcutta-1 variant in a higher percentage than the upper castes. In West Bengal, the Brahmins show a rate of 0.41 ± 0.26 per cent in contrast with 1.81 ± 0.44 percent in the lower caste group. In Tamilnadu, the Brahmin group shows a rate of 0.93 ± 0.53 per cent as against 2.98 ± 1.28 per cent in the Naicker and 4.14 ± 1.65 per cent in the Nadar groups. Das et al. (1972a) discussed this matter and clear evidences were brought out for a significant caste difference.

ORIGINATION OF CALCUTTA-1 VARIANT

This question was raised in Ananthakrishnan et al. (1970) and it was tentatively suggested if Calcutta-1 variant could enter India from the Middle East. The authors, however, did not maintain the idea as the expected clinal decline of the incidence rates of Calcutta-1 was not indicated by the existing data. So they hinted at the possibility of its origination in some Pre-Dravidian tribal group. But the recent finding of Undevia et al. (1972) among the Parsis showing about 3.6% incidence rate of Calcutta-1 variant in them seems very important in this connection.

The time seems not yet ripe to suggest a reasonable hypothesis. Further distribution data are very essential to form the basis of a hypothesis. Moreover, any hypothesis supposing its entry into India from outside must, first of all, establish that a variant exactly indentical with Calcutta-1 is found also in the proposed source. Although the Calcutta-1 variant is a faster A-subunit variant like the one detected in the Middle East (Turkish Cypriots) by Davidson et al. (1965), the two may not be identical. The Calcutta-1 variant has a peculiarity not reported for any other variant. The single LDH-1 band of Calcutta-1 (molecule B_4) should have a position in the zymogram exactly where it appears for the nodmal LDH. But, we

always notice a slight though definite anodal shift. No explanation could be offered for this phenomenon.

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