58,000 pc of I^{131} (0.058 μc), whereas an intake of 800,000 pc of I^{131} (1 liter of milk per day from our highest station) would deliver 14 rad. About 53,000 Utah children between 0 and 2 years of age were subject to I^{131} exposure in 1962 (from extrapolation of the 1950 and 1960 U.S. Census) (*9*).

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Lactate Dehydrogenase Variant from Human Blood: Evidence for Molecular Subunits

Abstract. *A variant of human lactate dehydrogenase is described. The occurrence of lactate dehydrogenase-1, -2, -3, and -4 as five, four, three, and two components, respectively, is interpreted as supporting the hypothesis that LDH isozymes are tetramers formed from various combinations of two types of subunits.*

The lactate dehydrogenase (LDH) of an organism may occur in multiple molecular forms called isozymes (*1*). LDH isozymes of approximately 135,000 molecular weight can be dissociated into subunits of 34,000 molecular weight (*2*). These subunits occur in two electrophoretically distinct forms (*2*) designated A and B. On the basis of this evidence several groups of investigators (*2, 3*) have suggested that distinction between individual isozymes arises, as shown in the second column of Table 1, from various tetrameric associations of A and B subunits. We have observed a variant of human LDH which supports this thesis.

The LDH variant appeared in a healthy 25-year-old Nigerian male of the Yoruba tribe who was initially examined during a survey for hemoglobin heterogeneity. Two separate samples of whole blood were obtained during a 2-month interval. Each sample was aseptically collected in acid-citrate-dextrose solution, sent to Baltimore, and received there 4 to 5 days later. Erythrocytes were washed in 0.9-percent NaCl, hemolyzed in water, extracted with toluene, and centrifuged at 40,000*g* for 20 minutes at 0°C. Hemolyzates containing approximately equal amounts of LDH were subjected to vertical starch-gel electrophoresis (*4*) (4°C, 4.5 volts/cm) for approximately 16 hours. After electrophoresis, LDH activity was localized by methods (*5*) modified from those of Dewey and Conklin (*6*).

As shown in Fig. 1 the variant specimen contains five components in the LDH-1 position, four in the LDH-2 position, three in LDH-3 position, and two in the LDH-4 position. At each isozyme position the most rapidly migrating variant component has the mobility of the corresponding isozyme from normal subjects. The LDH-5 component is absent in this and other hemolyzates (*7*). A similar, less intensely staining pattern is observed in plasma. The variant pattern is identical in separate blood specimens. Dilution of the sample had no effect on electrophoretic pattern. Other proteins present in the hemolyzate from the variant were indistinguishable from those of normal Negro subjects. The variant is demonstrable in gels prepared with many buffers including ethylenediaminetetraacetic acid-boric acid-tris (EBT) (*8*), borate (*4*), and the discontinuous system of Poulik (*9*). The mobilities and patterns of variant LDH-1 and LDH-2 are preserved after successive elution from starch gel, electrophoresis in starch granules (*10*), and repeated analysis in starch gel. Normal and variant LDH-1, isolated by successive starch gel-starch granule electrophoresis, possess similar Michaelis-Menten constants (K_m) for lactate (*10*) and nearly identical reaction rates with several nicotinamide adenine dinucleotide (NAD) analogs (*11*). For example, the reaction-rate ratio of the 3-acetylpyridine analog to the thionicotinamide analog of NAD was 0.15 for normal LDH-1 and 0.14 for variant LDH-1. In contrast, this ratio was 0.73 for normal LDH-5 from human skeletal muscle. The variant pattern is also detectable in LDH from erythrocytes which has

Table 1. Explanation for multiple components appearing within five major LDH zones.

Isozyme	Normal	Variant
LDH-1	B_4	B_4
		$B_3\beta_1$
		$B_2\beta_2$
		$B_1\beta_3$
		β_4
LDH-2	B_3A_1	B_3A_1
		$B_2\beta_1A_1$
		$B_1\beta_2A_1$
		β_3A_1
LDH-3	B_2A_2	B_2A_2
		$B_1\beta_1A_2$
		β_2A_2
LDH-4	B_1A_3	B_1A_3
		β_1A_3
LDH-5	A_4	A_4

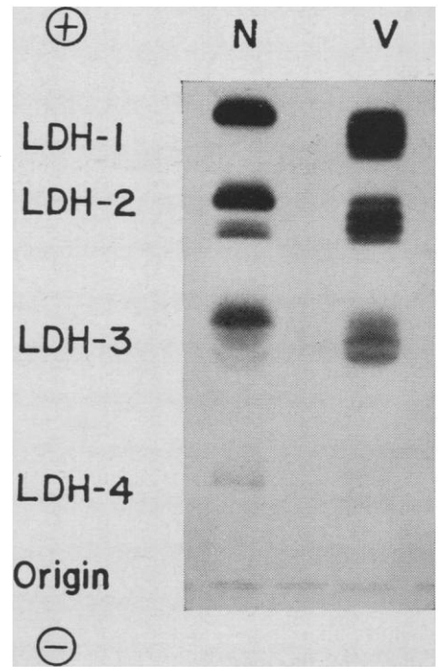

Fig. 1. Starch-gel electrophoretic pattern of normal (N) and human variant (V) LDH from erythrocyte LDH in EBT (*8*) buffer. The nature of the two bands lying just cathodic to normal LDH-2 is unknown (*5*). These components are uniformly present in normal hemolyzates but lacking in LDH of skeletal muscle. We observe such components in both horizontal and vertical starch-gel systems. A similar but very faint set of two bands lies midway between LDH-2 and LDH-3 in the variant hemolyzate. The LDH-3 components are slightly distorted by the coincident migration of hemoglobin A.

been purified approximately 300 fold by absorption and elution from diethylaminoethyl (DEAE) cellulose (*12*) followed by precipitation with 55-percent saturation of ammonium sulfate at 0°C. Moreover, when DEAE cellulose is developed with a linear NaCl gradient, the corresponding variant and normal LDH isozymes are eluted at similar points. The mobility of normal and variant LDH isozymes from crude hemolyzates is identical in the system (*13*) of varying starch-gel porosity. Such behavior suggests that the molecular weight of normal and variant isozymes is grossly similar (*14*). The enzymic activity of variant LDH from crude hemolyzate approximates that observed in normal American and Nigerian Negroes.

The only blood relative available for testing, a paternal uncle, has a normal LDH pattern. Whatever the origin of the observed LDH variation, it is uncommon (*15*) since no variant LDH patterns were observed among hemolyzates obtained from approximately 50 white subjects, 300 American Negroes, 100 Papuans (*16*), and 200 Nigerians.

Fritz and Jacobson (*17*) examined mouse tissue extracts and observed five LDH-5 components, four LDH-4 components, three LDH-3 components, two LDH-2 components, and one LDH-1 component in gels prepared with 0.005*M* β-mercaptoethanol. These authors suggest that this concentration of β-mercaptoethanol removes bound NAD from a portion of A subunits but not from B subunits. These observations provide a pattern exactly opposite to that in the Nigerian LDH variant. Moreover, the human variant LDH pattern is unaltered in gels containing β-mercaptoethanol in amounts ranging from 0.005 to 0.1*M*. The variant LDH pattern is also unaltered by electrophoresis under conditions where the gel and cathodic chamber contain $10^{-3}M$ NAD. Accordingly, we do not believe that the variant pattern is produced by the mechanism of differential binding of NAD.

The variant electrophoretic pattern can be most easily interpreted as reflecting a mutant allele at the genetic locus producing the LDH B subunit. The observed physical and enzymatic similarities between normal and variant LDH-1, that is, B subunit tetramers, support this interpretation. If the mutant product is designated β, and the normal product B, then a heterozygote at this locus should exhibit the combinations shown in the third column of Table 1. Such combinations will produce the pattern observed in Fig. 1 provided it is assumed that β is more electro-positive than B. Furthermore, if the mutant β and the normal B gene products are produced in equal quantity and thereafter randomly associate as tetramers, then LDH-1 of the heterozygote should contain B_4, $B_3\beta_1$, $B_2\beta_2$, $B_1\beta_3$, and β_4 in the binomial proportions 1:4:6:4:1. The proportions observed during enzyme development on starch gels roughly fit this ratio. Unfortunately diffusion of the localizing formazan dye prohibits satisfactory illustration. Such diffusion, close proximity of components, and the nonlinear relationship between dye intensity and enzyme concentration prevent exact spectrophotometric analysis. The expected fit is more evident among the other variant isozymes. By inspection, variant LDH-2 contains proportions which agree with a predicted 1:3:3:1. The components of variant LDH-3 and LDH-4 approximate the predicted ratios 1:2:1 and 1:1 respectively. LDH-5, if it were detectable, would be expected to be single and indistinguishable from the normal.

The variant LDH electrophoretic pattern is therefore consistent with the hypothesis that individual LDH isozymes are tetramers of two different subunits. The lack of demonstrable inheritance of the variant LDH necessarily limits this interpretation. A recent parallel to our observations is Markert's demonstration (*18*) that LDH-2, LDH-3, and LDH-4 can be generated in vitro by dissociation and subsequent reassociation of a mixture containing equal amounts of LDH-1 and LDH-5. In the reassociated mixture LDH-1, -2, -3, -4, and -5 occur in proportions 1:4:6:4:1. This is the same type of event which we believe has naturally occurred in vivo and produced five LDH-1 components from a mixture of mutant and normal B subunits (*19*).

Note added in proof: C. R. Shaw and E. Barto have described (*20*) inherited variation of LDH in *Peromyscus* which closely parallels the variant described here. Our interpretations are thereby corroborated.

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Sweating: Its Rapid Response to Muscular Work

Abstract. *Marked increases in sweating rate occurred within 1.5 seconds after the initiation of muscular activity in a warm room. This increase did not seem dependent upon thermal stimuli, but was related to the work rate, and the ambient temperature, or the sweating rate during the pre-exercise period.*

Although many studies have been devoted to the regulation of sweat production in the working human, no completely satisfactory explanation has evolved. Part of the reason for this may be that little attention has been given to reflex mechanisms which could be involved, especially in the