As mentioned, yeast RNA of high molecular weight is hydrolyzed at a rate about 5 percent of that of polyA. Soluble RNA, known to contain a high degree of secondary structure (*18*), is hydrolyzed at 1 percent of the rate of polyA hydrolysis. Indeed, the experiments on sRNA were carried out at the limit of detectability, and it may be that sRNA is not hydrolyzed at all by the purified enzyme. We have already mentioned that polyI is resistant to hydrolysis. In addition, polyUG containing 0.58 mole of uracil to 1 mole of guanine is resistant to hydrolysis. The properties of this polymer have been described (*19*), and it is known to contain a high degree of secondary structure.

The data presented in Table 1 indicate that the addition of helical polynucleotides to reaction mixtures does not inhibit the breakdown of single-stranded polyribonucleotides. Thus, polyI which is itself resistant to enzymic attack does not affect polyU hydrolysis, and sRNA does not affect polyA hydrolysis. In addition, native DNA is not inhibitory. Actually, this finding can also be inferred from the data in Fig. 1. In the experiment shown in the upper curve, the concentration of free polyA should be 0.55 μmole per milliliter of reaction and the rate of hydrolysis is 24 μmole per hour per milliliter of enzyme. A separate experiment, in which we studied the rate of polyA hydrolysis as a function of polyA concentration, gave just such a rate at 0.55 μmole of polyA per milliliter of reaction mixture.

The evidence indicates that the potassium-activated phosphodiesterase of *E. coli* is unable to degrade helical polyribonucleotides. In this sense it differs from the ribosome-bound ribonuclease of *E. coli* (*7, 20*) but is similar to polynucleotide phosphorylase (*5*). As suggested by Spahr and Schlessinger (*6*) therefore, this enzyme, as well as polynucleotide phosphorylase, may be responsible for the rapid breakdown in vitro of messenger RNA that has been noted by others (*21*). The mechanism by which the enzyme functions in vivo, particularly with respect to messenger RNA bound to ribosomes, remains unclear. The binding of the enzyme to ribosomes is of interest from this point of view.

This diesterase should be a useful tool for studying both the primary and secondary structure of polyribonucleotides. The absence of detectable phosphomonoesterase and polynucleotide phosphorylase from the purified fractions enhances this utility. The apparent lability of the purified enzyme will be a disadvantage unless stabilizing conditions can be found. It should perhaps be pointed out that this enzyme appears to be distinct from the *E. coli* phosphodiesterase described by Lehman (*2*). That enzyme, although it also produces 5′-mononucleotides and prefers single-stranded polynucleotides, is specific for polydeoxyribonucleotides, and has a *p*H optimum of about 9.

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References and Notes

1. Abbreviations: 5′-AMP and 5′-UMP refer to 5′-adenylic and 5′-uridylic acids, respectively; DNA is deoxyribosenucleic acid and RNA is ribosenucleic acid. In addition, sRNA indicates transfer (soluble) RNA; and the capital letters A, U, C, G, and I are used for the nucleotides adenylic, uridylic, cytidylic, guanylic, and inosinic acids, respectively, when they are residues in a polyribonucleotide chain (for example, polyA is polyriboadenylic acid); also OD, optical density; DEAE, diethylaminoethyl.
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Human Erythrocyte Lactate Dehydrogenase: Four Genetically Determined Variants

Abstract. *Four variants of human erythrocyte lactate dehydrogenase (LDH) are described. One of these is similar to the one previously described by Boyer and is interpreted as a variant of LDH-B. The other three are thought to represent variants of LDH-A. Studies of families indicate that LDH-A and LDH-B are under separate genetic control and are inherited as autosomal co-dominants. Evidence is presented that the LDH-A locus is not closely linked to the loci for the ABH and Duffy blood group systems.*

Lactate dehydrogenase (LDH) is an enzyme which has a molecular weight of approximately 135,000 and which can be dissociated into four subunits of equal size (*1*). Electrophoresis on starch granules, starch gels, or acrylamide gels separates LDH into five distinct isozymes (*2*). It has been suggested that LDH exists in two electrophoretically distinguishable forms, LDH-A and LDH-B, and that the five isozymes are tetramers formed by random association of the LDH-A and LDH-B subunits—that is, they have the following composition: LDH-1, A_0B_4; LDH-2, A_1B_3; LDH-3, A_2B_2; LDH-4, A_3B_1; and LDH-5, A_4B_0 (*1*).

The relative amounts of the isozymes vary with the tissue and species studied. In the lactate dehydrogenase of erythrocytes the LDH-B subunits predominate so that on electrophoresis the LDH-4 zone is faint and the LDH-5 band is absent (*3*). Boyer *et al.* (*4*) have observed a variant pattern of erythrocyte LDH in an individual from Nigeria; Nance *et al.* (*5*) have presented evidence for another erythrocyte LDH variant. We have studied the LDH isozyme patterns of

erythrocytes from 940 randomly selected hospital employees (610 Negroes and 330 Caucasians) and found eight persons with variant patterns. The patterns fell into four distinct groups. Pedigrees of three of these individuals are presented.

For screening purposes hemolysates were prepared from unwashed, sedimented erythrocytes with the aid of chloroform. Hemolysates were fractionated by vertical starch gel electrophoresis (*6*) at 4°C with tris-EDTA-borate buffer (*7*), at *p*H 8.6. A current of 8 v/cm was applied for 14 to 16 hours, during which time hemoglobin A migrated to about 10 cm from the origin. After separation, gels were sliced and sections 3 mm thick were stained for LDH activity with phenazine methosulfate and nitro blue tetrazolium at *p*H 7.4 (*8*). Variant patterns were confirmed by examining fresh hemolysates from thrice-washed erythrocytes lysed either with a mixture of toluene and distilled water, or by the addition of distilled water followed by repeated freezing and thawing. The LDH patterns of fresh hemolysates were not altered by addition of nicotinamide-adenine dinucleotide to the starch gel and buffer (*5*).

Four different variants of erythrocyte LDH isozymes were found in seven Negroes and one Caucasian out of 940 individuals studied (0.9 percent). The LDH variants were named LDH$_{Mem-1,-2,-3 and -4}$ (Mem indicating Memphis) (Fig. 1) in order of their discovery, following the pattern established for temporary identification of abnormal hemoglobins. LDH$_{Mem-3}$ appears to be similar to the variant found by Boyer *et al.* (*4*). The isozyme LDH-1 of this variant can be resolved into five components with the fastest moving component in the position of normal LDH-1. The isozymes LDH-2, LDH-3, and LDH-4 of the variant are resolved into four, three, and two components, respectively. The LDH-5 band is absent in this and other hemolysates. These findings agree with the hypothesis (*4*) that individuals with this variant possess a normal LDH-A, but are heterozygous for LDH-B and a LDH-B variant (B′). We, therefore, designate this LDH variant as LDH-B$_{Mem-3}$.

The LDH-1 band of LDH$_{Mem-1,-2, and -4}$ appears in the normal position. The LDH-2 of LDH$_{Mem-1}$ consists of two components, one in the location of normal LDH-2, the other moving slightly more toward the anode. The LDH-3 of this variant consists of three bands; LDH-4 is not clearly shown.

In LDH$_{Mem-2}$, LDH-2 also consists of two components, but differs from LDH$_{Mem-1}$ in that the second component migrates even further toward the anode than the corresponding component of LDH$_{Mem-1}$. LDH-2 and LDH-3 of LDH$_{Mem-2}$ again consist of two and three components, respectively, though more widely spaced than those of LDH$_{Mem-1}$.

The isozyme LDH-1 is also in the normal location in LDH$_{Mem-4}$. The LDH-2 of this variant is divided into two components. One of these has the same electrophoretic mobility as normal LDH-2, the other moves more slowly toward the anode and therefore lies closer to the origin. The LDH-3 of LDH$_{Mem-4}$ divides into three components, the most rapidly migrating one lying in the position of normal LDH-3. LDH-4 is not clearly shown and LDH-5 is again absent.

The LDH activity of erythrocytes was estimated quantitatively at 37°C and *p*H 7.5 (*9*) and related to the total red cell count, hemoglobin, and hematocrit values. No significant difference was noted in the four variants of the enzyme as compared with normal LDH. The serums of all persons with variant LDH patterns were subjected to starch gel electrophoresis and all showed the same isozyme variants as erythrocytes.

We suggest that the abnormalities in LDH$_{Mem-1,-2, and -4}$ lie in the LDH-A moiety, LDH-B being normal, and we designate them as LDH-A$_{Mem-1,-2, and -4}$. In LDH-A$_{Mem-1}$, LDH-A and a slightly more negatively charged LDH-A′ combine with a normal LDH-B thereby causing the formation of two components of LDH-2, three of LDH-3 and presumably four of LDH-4 and five

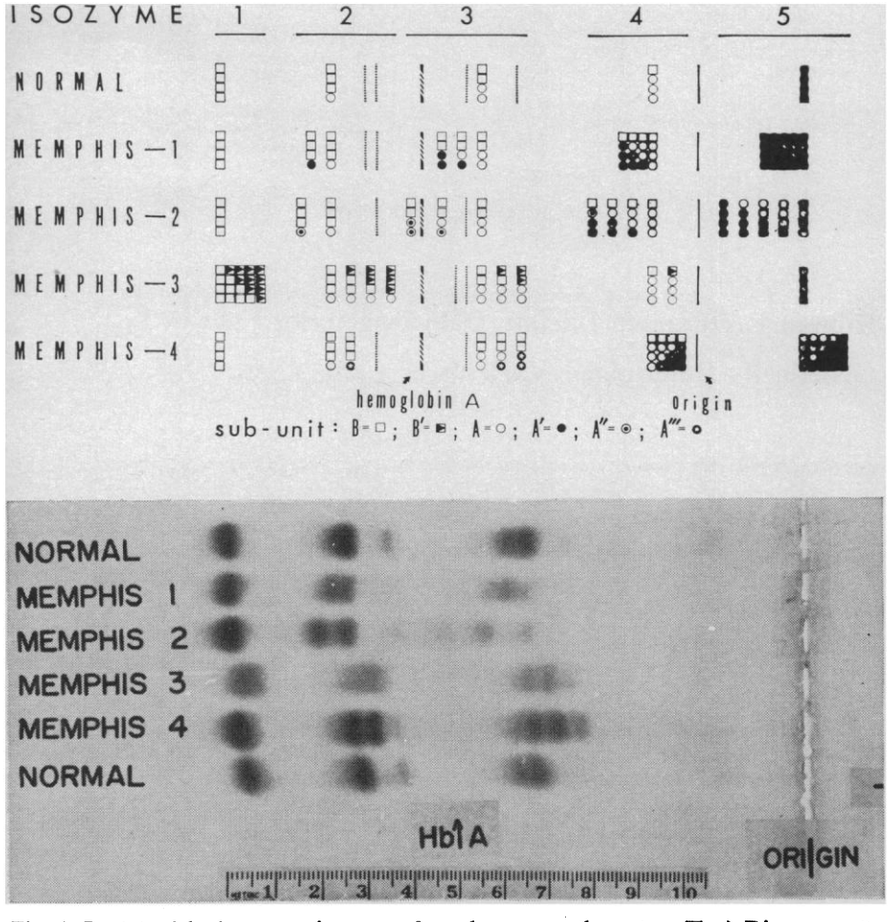

Fig. 1. Lactate dehydrogenase isozymes from human erythrocytes. (Top) Diagrammatic representation. Shaded areas are hypothetical and were not shown on the starch gels. The dotted lines in LDH-2 and LDH-3 indicate bands with enzyme activity seen on most of the gels. These bands tend to disappear in higher concentrations of buffer and were therefore considered to be artifacts in vitro (*5, 9*). (Bottom) Vertical starch gel electrophoresis, *p*H 8.6, tris-EDTA-borate buffer (*7*). The four variant enzyme patterns are shown.

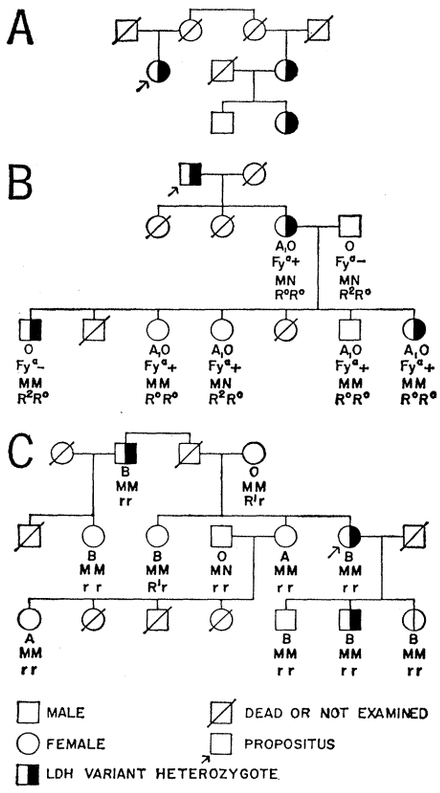

Fig. 2. Pedigrees of families with LDH variants. (*A*) LDH-A_{Mem-2}. (*B*) LDH-B_{Mem-3}; all family members examined were Kell negative and Sutter *a* and *b* positive. (*C*) LDH-A_{Mem-4}; all family members examined were Kell negative.

of LDH-5. The formation of the isozyme bands of LDH-A_{Mem-2} is explained in a similar fashion, except that the altered LDH-A, that is, A″, of this variant moves further toward the anode than that of LDH-A_{Mem-1}. LDH-A_{Mem-4} also consists of a normal LDH-B moiety, but is formed by a normal LDH-A and an altered, more positively charged LDH-A‴. The additional isozyme components of this variant therefore lie closer to the origin in starch gel electrophoresis than the corresponding normal isozymes.

Fritz and Jacobson (*10*) suggested that each unit of LDH-A and LDH-B has attached to it a molecule of nicotinamide-adenine dinucleotide (NAD). These authors postulated that 0.005*M* β-mercaptoethanol partially removes NAD from LDH-A, but not from LDH-B. Mouse tissue extracts thus treated show LDH-5 to be broken up into five components, four components for LDH-4, three for LDH-3, and two for LDH-2. LDH-1 is not altered since it consists of LDH-B units. These findings are reminiscent of the patterns shown by LDH-$A_{Mem-1, -2, and -4}$. However, we do not believe that partial dissociation of LDH-A and NAD play any role in the formation of the variant LDH patterns because (i) the addition of NAD to the starch gel and buffer did not alter the pattern; (ii) electrophoresis of freshly prepared hemolysates of the affected individuals always resulted in identical abnormalities, and these were never seen in normal persons; (iii) all affected relatives of affected individuals always possessed an identical abnormality.

The erythrocyte LDH variant described by Nance *et al.* (*5*) has a fast moving LDH-2 component as observed in LDH-$A_{Mem-1 or -2}$, but without direct comparison identity cannot be established. These authors report that the LDH-3 isozyme of their patient separated into only two bands with the band containing the mutant LDH-A migrating just behind hemoglobin A. As shown in Fig. 1, LDH-3 of LDH-$A_{Mem-1 and -2}$ clearly separates into three bands, the middle band staining more intensely than the other two. This finding supports the concept of random association of LDH-A, variant LDH-A, and LDH-B subunits, a process which in LDH-3 would lead to the formation of three bands in the proportions of 1 : 2 : 1. Boyer *et al.* (*4*) have shown that this indeed occurs in an individual heterozygous for LDH-B and for a variant LDH-B, and it would be reasonable to expect the same phenomenon in persons with an LDH-A variant.

LDH-A_{Mem-1} was found in three Negroes, one of whom had sickle cell trait (Hb A-S). Family studies could not be performed. LDH-A_{Mem-2} was found in two Negroes, one of whom also had the high fetal hemoglobin trait. His family unfortunately was not available for study. The pedigree of the other family with LDH-A_{Mem-2} is shown in Fig. 2*A*. The family of one of the two Negroes with LDH-B_{Mem-3} was studied and is shown in Fig. 2*B*. LDH-A_{Mem-4} was found in a single Caucasian and her family (Fig. 2*C*). All affected individuals of one family possessed the same erythrocyte LDH variant. The LDH variants appeared to be inherited as autosomal codominant characteristics. All the individuals found are considered to be heterozygotes either for LDH-A or for LDH-B. The study of the family with LDH-B_{Mem-3} indicates that there is no linkage of the locus for LDH-B with either the ABH system or the Fy^a gene. Hemoglobin electrophoresis and blood typing for the Rh system and the MNS, Kell, and Sutter groups were performed on all family members, but the results do not permit any conclusions as to linkage between those characteristics and the LDH loci. The finding of eight individuals with variant LDH enzyme patterns in a group of 940 randomly selected persons indicates that this variation may not be as rare as heretofore suspected.

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Myosin Substructure: Isolation of a Helical Subunit from Heavy Meromyosin

Abstract. *A highly α-helical subunit has been isolated from the heavy-meromyosin portion of myosin. This molecule has a helix content of about 73 percent and an amino acid composition similar to that of light meromyosin. The presence of this subunit supports the current view that the myosin molecule consists of a long helical rod with a globular region at one end.*

Muscle fibers owe their striation to two sets of interdigitating filaments: the thick, or myosin-containing filaments, and the thinner, actin-containing filaments (*1*). Interactions between the filaments seem to be mediated by projections or "bridges" periodically arranged along the myosin filaments. In