fragments recorded for α-MNE–PFP and α-MDA–PFP were the same: *m/e* 190 and *m/e* 428, respectively. Because of unavoidable differences between two analyses, it was necessary to use the internal standards to normalize the values for DA-PFP and NE-PFP. In this way, the ratio for two fragments analyzed for endogenous NE-PFP (*m/e* 176/590) and DA-PFP (*m/e* 428/176) compared with the ratios obtained from pure standards analyzed in the same way and with peak ratios calculated from the mass spectra (Table 1). Tissue samples were also processed without internal standards to ascertain that the fragments measured for the internal standards originated from the α-MNE and α-MDA added to the tissue sample and were not "biological background."

To demonstrate the practical significance of this method, we measured the NE and DA content in various tissues (Table 2). The data show that the concentrations of catecholamines detected with mass fragmentography compare with the reported available data. The true value of this method is that it can be applied to the study of catecholamines in very discrete brain structures weighing less than 100 μg (Table 2).

Thus the combined techniques of GC-MS can be used to quantitatively analyze 10^{-13} to 10^{-14} moles of endogenous amines. The compounds are separated by the GC and measured with the MS. The assay procedure is relatively rapid and we can process 15 to 20 samples in a day's work, including their dissection and the necessary standard curves. Considering the specificity given by the GC retention time and, more important, from the ion density recorded at a specific *m/e* setting during the elution of the various compounds from the GC, the specificity of this method surpasses and cannot be compared to any other existing quantitative method.

S. H. KOSLOW
F. CATTABENI
E. COSTA
Laboratory of Preclinical Pharmacology, National Institute of Mental Health, Saint Elizabeths Hospital, Washington, D.C. 20032

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8. In the simultaneous recording of different fragments, the accelerating voltage alternator is used. The LKB 9000 GC-MS requires that the peaks to be recorded are within 10 percent of each other (*m/e*). Since NE-PFP and DA-PFP do not have abundant fragments within 10 percent of each other, it was not possible to do conventional multiple ion detection.
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Genetic Control of Lactate Dehydrogenase Expression in Mammalian Tissues

Abstract. *The amount of lactate dehydrogenase isozyme 4 in erythrocytes of mice is controlled by alleles at the* Ldr-1 *locus. The A subunits of lactate dehydrogenase from erythrocytes deficient in isozyme 4 cannot assemble in vitro with B subunits to form active isozyme. The inability to form hybrid enzyme is not due to a mutation in the structural gene for the A polypeptide. Rather, a factor that is bound to the A subunits of erythrocytes restricts free exchange with B subunits.*

The concentration of an enzyme in a mammalian cell is probably regulated at the level of synthesis or intracellular degradation (*1*). However, for those enzymes composed of more than one polypeptide chain, another level at which regulation could occur is the assembly of subunits to form the active enzyme. Rosenberg (*2*) showed that the two subunits of lactate dehydrogenase (LDH) in fish probably combine in vivo in a nonrandom fashion to form tissue-specific isozyme patterns, a result that suggests that the primary structures of the subunits are not the only determinants of the quaternary structure of the active enzyme. Fritz *et al.* (*3*) also proposed that the changes in rat heart LDH isozymes during development are regulated by events after synthesis of the polypeptide chains. If the quaternary structure of an enzyme is determined both by the primary structures of its polypeptides and by the environment in which these polypeptides assemble, then mutations that alter the environment could affect the expression of the enzyme. In this report we present evidence that the pattern of LDH isozymes in erythrocytes in mice is regulated by a factor (or factors), under genetic control, that affects the assembly of LDH subunits.

Mammalian LDH is a tetramer of two dissimilar subunits, A and B, which assemble in all possible combinations to form five isozymes. Isozymes LDH-1, LDH-2, LDH-3, LDH-4, and LDH-5 have subunit compositions of B_4, B_3A_1, B_2A_2, B_1A_3, and A_4, respectively. These isozymes differ in charge and can be separated by electrophoresis (Figs. 1 to 3) or ion-exchange chromatography (*4*).

Shows and Ruddle (*5*) described a variant of the LDH pattern in mice. Erythrocytes of most inbred strains contained only LDH-5, but erythrocytes of strains SWR/J, DE/J, LP/J, and DW/J contained both LDH-5 and LDH-4. Thus, erythrocytes of the latter strains contained both A and B subunits, but erythrocytes of all other strains contained only A subunits. The tissue-specific isozyme patterns in all other tissues were identical among the strains. The erythrocyte pattern is inherited as a single Mendelian factor and is under the control of alleles at a locus *Ldr-1*, which is on linkage group XI (*6*).

Figure 1 shows the LDH isozymes from erythrocytes of mice homozygous for alternate alleles at the *Ldr-1* locus. Isozyme LDH-4 is deficient in the homozygous *Ldr-1*^{a} strain, C57BL/6J. When the LDH isozymes in erythrocytes were separated on diethylaminoethyl cellulose (DEAE-cellulose), LDH-4 was about 1 percent of the LDH activity in the C57BL/6J strain; in contrast, this isozyme was about 4 percent of the LDH activity in the homozygous *Ldr-1*^{b} strain, SWR/J. The tissue-specific isozyme contents of kidney, liver, and heart of the two strains was identical. Thus, the defect in LDH-4 expression is restricted to the erythrocyte.

A possible explanation for the tissue-specific effect of mutation at the *Ldr-1* locus is that this locus controls

a factor (or factors) in the erythrocyte, and that the factor in the *Ldr-1*^{a} strain restricts assembly of A and B subunits to form LDH-4. We examined this possibility with the technique of molecular hybridization in vitro.

In dissociation and recombination studies (*7*), LDH-5 (A_4) from erythrocytes of SWR/J mice—the isozyme either in hemolyzates or partially purified—was tested with LDH-1 (B_4) from kidneys of either SWR/J or C57BL/6J mice. Five isozymes were formed, as predicted from random assembly of subunits (Fig. 2). Conversely, LDH-5 from C57BL/6J erythrocytes, in hemolyzates or purified 70-fold, did not combine with LDH-1 from either strain, regardless of the quantity of B subunits available for hybridization (Fig. 2, d and f). The inability of LDH-5 from C57BL/6J erythrocytes to hybridize is probably not due to a mutation in the structural gene for the A polypeptide. Isozymes LDH-1, LDH-3, and LDH-5 were partially purified from kidney, and LDH-5 was partially purified from erythrocytes; corresponding isozymes from the two strains were indistinguishable as judged by electrophoretic mobility in polyacrylamide gels, stability to heat, and stability to denaturation with urea. Isozyme LDH-5 from erythrocytes appeared identical to the same isozyme from kidney by these criteria. A mutation in the polypeptide chain would be expected to affect the A subunits of every tissue. The isozyme pattern was normal in all tissues except erythrocytes. Furthermore, when A and B subunits from other tissues of both strains were dissociated and recombined, five isozymes were formed, a result that indicates that the *Ldr-1* locus affects the A subunit of only the erythrocyte.

Isozyme LDH-5 from C57BL/6J erythrocytes inhibits the ability of LDH-5 from SWR/J erythrocytes to associate with LDH-1 (Fig. 3, a and b). The factor restricting association of subunits is purified along with LDH-5 from C57BL/6J erythrocytes through ion-exchange chromatography, $(NH_4)_2SO_4$ fractionation, and chromatography on Sephadex G-100. However, the factor can be released from LDH-5 by heat denaturation of the isozyme. The supernatant fraction after heat treatment contains no LDH activity but prevents the A subunits of SWR/J erythrocytes from hybridizing with kidney B subunits (Fig. 3c).

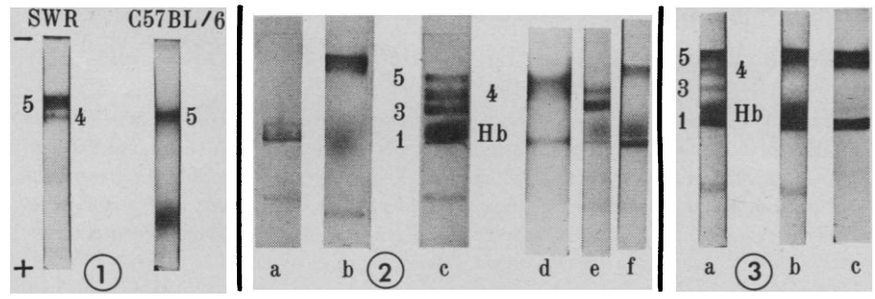

Fig. 1. Lactate dehydrogenase isozymes in erythrocytes of SWR/J and C57BL/6J mice. Hemolyzates were subjected to electrophoresis in polyacrylamide gels (*10*), and the LDH isozymes were localized as described by Vesell and Bearn (*11*). Activity was assayed as described by Markert and Faulhaber (*12*). The gel containing C57BL/6J hemolyzate was overloaded in an attempt to demonstrate LDH-4. The protein band toward the anode is hemoglobin. Fig. 2. Dissociation and recombination of LDH isozymes. Kidney LDH-1 from both strains was separated from the other isozymes by DEAE-cellulose chromatography of a 105,000*g* supernatant fraction. Isozyme LDH-5 from erythrocytes of both strains was partially purified by DEAE-cellulose chromatography, $(NH_4)_2SO_4$ fractionation, and Sephadex G-100 chromatography. Samples are (a) LDH-1 from kidney of strain SWR/J; (b) LDH-5 from erythrocytes of strain SWR/J; (c) 80 units of SWR/J LDH-1 hybridized with 80 units of SWR/J erythrocyte LDH-5; (d) 60 units of SWR/J LDH-1 hybridized with 60 units of C57BL/6J LDH-5; (e) 160 units of C57BL/6J kidney LDH-1 hybridized with 80 units of SWR/J erythrocyte LDH-5; and (f) 160 units of C57BL/6J kidney LDH-1 hybridized with 80 units of C57BL/6J erythrocyte LDH-5. Each hybridization mixture contained 1*M* NaCl and was either frozen once in Dry Ice or twice at −15°C (*Hb*, hemoglobin). Fig. 3. The presence of a factor restricting subunit association in C57BL/6J erythrocytes. Hybridization mixtures a and b contained 80 units of SWR/J kidney LDH-1. In addition, mixture a contained 80 units of SWR/J erythrocyte LDH-5. Mixture b contained 40 units each of SWR/J and C57BL/6J erythrocyte LDH-5. Mixture c contained 60 units of both erythrocyte LDH-5 and kidney LDH-1 from the SWR/J strain plus the supernatant (0.4 ml) obtained after heating a preparation of C57BL/6J erythrocyte LDH-5 for 10 minutes at 100°C (*Hb*, hemoglobin).

The subunits of LDH apparently exchange among the different isozymes in situ (*3*, *8*). Our results suggest that a factor bound to the A subunits of LDH in erythrocytes of C57BL/6J mice prevents the A subunits from associating with B subunits. Thus, during the normal course of association and disassociation of erythrocyte LDH-5, the A subunits cannot freely combine with B subunits to form isozyme 4, although B subunits are present in the erythrocyte.

The factor bound to the A subunits of C57BL/6J erythrocytes has not been identified, but it has many of the properties of the derivative of reduced nicotinamide adenine dinucleotide which was described by Gelderman and Peacock (*9*). This derivative also binds to LDH-5; it is difficult to remove by ion-exchange chromatography, $(NH_4)_2SO_4$, or dialysis; and it is stable to heat. Whatever the nature of the factor restricting subunit assembly, the amounts of the individual LDH isozymes in the erythrocyte do not result from a random tetramerization of the two subunits. Rather, the quaternary structure of LDH is determined at least in part by tissue-specific mechanisms. These mechanisms are under genetic control but are not related to the structural genes for the A and B polypeptides or directly to the rates of subunit synthesis and degradation.

RICHARD D. GLASS
DARRELL DOYLE
Department of Anatomy and Cell Biology, University of Pittsburgh Medical School, Pittsburgh, Pennsylvania 15213

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