uremic control group. Further purification of the Ca BP with Sephadex G-100 chromatography revealed that the Ca BP activity in both normal and uremic rats was associated with a specific protein fraction and therefore was not a general property of all the proteins contained in the crude heat-treated supernatant (Fig. 1). This isolation procedure resulted in a protein fraction (tubes 140 to 155) which contained all the Ca BP activity in the mucosa homogenates of untreated animals as well as from those treated with vitamin D_3 and 25-hydroxycholecalciferol.

Like that of the crude heat-treated supernatant from mucosa homogenates of uremic animals (Table 2), the binding activity of material isolated by Sephadex chromatography rose minimally after vitamin D_3 treatment but more than twofold after administration of the 25-hydroxycholecalciferol vitamin D_3 metabolite. Because 10 to 16 hours may be required before the defective intestinal calcium absorption is measurably restored to normal by oral vitamin D_3 therapy (*10*), calcium-binding activity of duodenal mucosa was also measured in uremic rats 24 and 48 hours after 500 units of vitamin were administered. In each instance, no significant increment in Ca BP was demonstrable when compared to the Ca BP activity of uremic rat mucosa obtained 12 hours after vitamin D_3 administration.

So far, vitamin D dependent Ca BP has been detected in the intestinal mucosa of the rat, dog, and monkey (*11*). The nature of the interaction of vitamin D with the mucosal cell that leads to the formation of the calcium-binding protein is still in doubt. Studies by Zull, Czarnowska-Misztal, and De Luca (*12*) and Norman (*13*) suggest that this interaction represents either a direct effect of 25-hydroxycholecalciferol on the DNA–messenger RNA complex of the mucosal cell or an indirect alteration of the permeability characteristics of the nuclear membrane. Although the product of this nuclear or membrane interaction has been proposed to be a translocase or transport enzyme (*12*), the possibility that the substance formed is identical to the vitamin D dependent Ca BP isolated from the chick mucosa by Wasserman and co-workers (*5, 7, 9, 11*) must be entertained. Should this interpretation prove correct, alterations in vitamin D_3 metabolism in uremia (*3*) resulting in decreased intestinal 25-hydroxycholecalciferol concentrations could lead sequentially to decreased synthesis and concentration of the intestinal Ca BP and contribute to the defective calcium absorption characteristic of the chronic uremic state.

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Homology of Lactate Dehydrogenase Genes: E Gene Function in the Teleost Nervous System

Abstract. *Data obtained from immunochemical studies and the use of allelic variants support the hypothesis that the lactate dehydrogenase isozymes unique to the teleost nervous system are encoded in a third locus. Immunochemical and other studies demonstrate that this third locus is closely related to the lactate dehydrogenase B locus.*

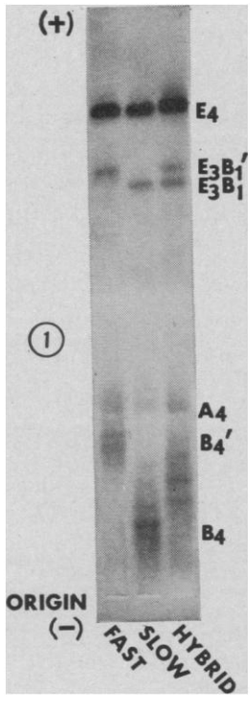
Lactate dehydrogenase (LDH) exists in several isozymic forms in many organisms (*1*). The LDH polypeptide subunits in vertebrates are encoded in at least two codominant loci (*2, 3, 4*). The two corresponding types of polypeptide subunits produced are designated A and B (*2*). These subunits usually self-assemble randomly in vivo (*2, 4, 5*) or in vitro (*6*) to form the five tetrameric isozymes A_4, A_3B_1, A_2B_2, A_1B_3, and B_4 which are readily separable by electrophoresis.

Mammals and birds possess three LDH loci. The third locus, the C locus, functions only in primary spermatocytes (*7, 8, 9*). A third locus, referred to as the E locus (*10*), has also been postulated for teleosts by Markert and Faulhaber (*11*). The E locus, like the C locus, also exhibits great specificity of cell function (*10–16*). The E_4 isozyme, encoded in the E locus, usually possesses a very high net negative charge and is localized in the nervous system of teleosts, especially in the retina (*10–17*). However, in some species of teleosts the E_4 isozyme is also found in nonneural tissues such as lens (*12, 17*). On the basis of a comparison of the kinetic and physical properties of the teleost isozymes, I propose that the E_4 isozyme may be more closely related to the B_4 than to the A_4 isozyme (*17*).

The E subunits differ from the A and B subunits by hybridization in vivo (*10–14*) and in vitro (*10, 12, 14*). Especially revealing are the two types of E subunits produced in hybrid trout which are progeny of different parental species (*16*). Nevertheless these observations on subunit polymerization do not permit a critical conclusion concerning the genetic or epigenetic basis for the difference in subunit properties.

In order to clarify the genetic origin of the E subunit, allelic isozyme variants at the LDH B locus were examined in a panmictic population of killifish (*Fundulus heteroclitus*) from Woods Hole, Massachusetts. Figure 1 shows the LDH isozyme patterns of killifish eyes. The isozyme unique to the eye (E_4) migrates most rapidly toward the anode. The reversal of the relative electrophoretic mobility commonly observed in the A_4 and B_4 isozymes does not significantly alter their kinetic, physical, and immunochemical properties, which are homologous with those of the A_4 and B_4 isozymes of other vertebrates.

The three LDH phenotypes (Fig. 1) and their underlying genotypes responsible for alteration of B subunit mobility are slow (BB) and fast (B′B′), the two homozygotes, and the heterozygous hybrid (BB′) which possesses isozymes of intermediate mobility. The diallelic basis proposed for these phenotypes is supported by a population analysis of 245 adult killifish. The gene frequencies of the two *B* alleles were estimated



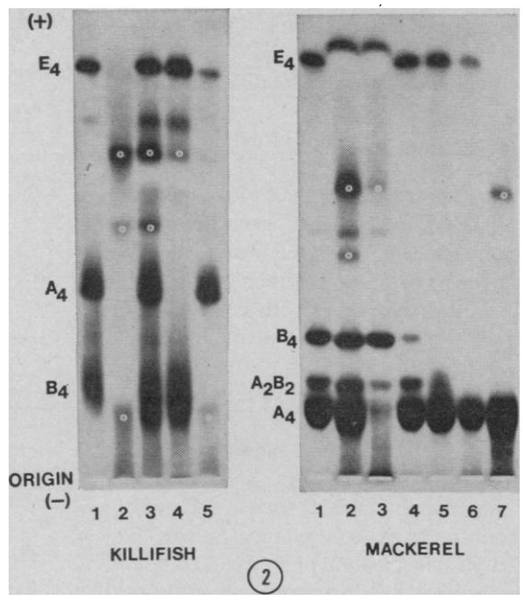


Fig. 1. Lactate dehydrogenase isozyme phenotypes of killifish (*Fundulus heteroclitus*) eyes. Electrophoresis was at 10 volt/cm for 16 hours at 4°C in a 13 percent, vertical starch gel in the discontinuous buffer system of Boyer *et al.* (*28*). The only subunits exhibiting genetic variation are the B subunits. *Fast* is the B'B' homozygote; *slow* is the BB homozygote; *hybrid* is the B'B heterozygote. Some of the minor multiple bands appear to represent subbanding because their position is altered as a unit because of the allelic variation. Fig. 2. Effect of B_4 and A_4 antiserums upon the lactate dehydrogenase isozymes from the eyes of killifish (*Fundulus heteroclitus*) and mackerel (*Scomber scombrus*). The antiserums were mixed in various concentrations with the eye isozymes for 2 hours at 4°C and centrifuged at 105,000*g* for 40 minutes at 4°C; the supernatant was then subjected to electrophoresis as in Fig. 1. The isozyme bands marked with a small white circle are the LDH isozymes present in the serum. The mackerel has only isozymes A_4, A_2B_2, and B_4 in the nonneural tissues. Killifish: (1) untreated eye LDH isozymes; (2) antiserum alone; (3) eye LDH isozymes plus normal serum [1:1]; (4) eye LDH isozymes plus A_4 antiserum [1:1]; (5) eye LDH isozymes plus B_4 antiserum [5 : 1]. Mackerel: (1) untreated eye isozymes; (2) eye LDH isozymes plus normal serum [1:1]; (3) eye LDH isozymes plus A_4 antiserum [1:1]; (4) eye LDH isozymes plus B_4 antiserum [20:1]; (5) eye LDH isozymes plus B_4 antiserum [10:1]; (6) eye LDH isozymes plus B_4 antiserum [3:1]; (7) eye LDH isozymes plus B_4 antiserum [1:1].

at B' equal to 0.269 and B equal to 0.731. These gene frequencies were used in the Hardy-Weinberg formula to generate the expected phenotypic frequency of slow equal to 130.8, hybrid equal to 96.4, and fast equal to 17.8 which is not significantly different from the observed phenotypic frequencies of slow equal to 134, hybrid equal to 90, and fast equal to 21 (chi-square = 1.09 and $P \cong 0.6$). All phenotypes were evenly distributed among males and females.

Neither the E_4 isozyme nor the A_4 isozyme is shifted in electrophoretic mobility even though the B subunit exhibits altered mobility due to mutation (Fig. 1). The hybrid isozymes formed as a result of association of E and B subunits, for example, E_3B_1 and $E_3B'_1$, reflect in their number and electrophoretic mobility the subunit contributions of the two different *B* alleles. Thus, it is very unlikely that the E_4 isozyme can be the result of an epigenetic alteration of the B subunit. An alternative explanation which has not been completely excluded is that the amino acid substitution in the mutant B polypeptide occurs in a region that is eliminated or modified during epigenetic conversion of B subunits to E subunits. This point of view appears unlikely on two grounds. First, mutations at the B locus in other species of fish do not alter the E subunit (*13, 18*); second, I have found two killifish which appear to be heterozygous at the E locus and which possess electrophoretic variants of the E_4 isozyme. The mobility of this E_4 isozyme and the hybrid isozymes containing E subunits are altered without any change in the mobility of the B_4 or A_4 isozymes. Because genetic alteration of B or E subunits alters only the behavior of the corresponding subunit, I conclude that all three subunits, A, B, and E, are under separate genetic control. Immunochemical data further support this conclusion.

The A_4 and B_4 isozymes of weakfish (*Cynoscion regalis*) were purified, and antibodies to each homopolymer were induced in rabbits (*19, 20*). The precipitating equivalence point for the B_4 antibodies is 135 international units of B_4-LDH per milliliter of antiserum, and that for the A_4 antibodies is 60 international units of A_4-LDH per milliliter of antiserum. The antiserum to the weakfish A_4 and B_4 isozymes does not cross-react with the other homopolymer, and this agrees with the immunochemical distinctiveness of the A_4 and B_4 isozymes in other vertebrates (*18–20*). Antiserum to either of the weakfish isozymes cross-reacts well with the homologous isozyme from different groups of teleosts such as killifish and mackerel (*Scomber scombrus*). The A_4 or B_4 antiserum was mixed with the LDH isozymes of the eye for 2 hours; then, after centrifugation, the supernatant was examined by electrophoresis. The reduction or absence of specific isozyme bands on the gel after electrophoresis is a dramatic illustration of their immunochemical similarity. In both the killifish and mackerel the A_4 antiserum reacts only with those isozymes containing A subunits and not with the B_4 or E_4 homopolymers, even at high concentrations of antiserums (Fig. 2). Thus the A subunits are immunochemically distinct from B and E subunits, which is additional evidence for their separate genetic control. The B_4 antiserum in relatively low concentrations reacts appreciably only with isozymes containing the B subunit.

Additional physical evidence suggesting a genetic difference between the E subunits and the A and B subunits is provided by molecular hybridization studies. In some teleosts, weakfish for example, the A and B subunits do not associate with each other either in vivo or in vitro, yet the E subunit can associate with either A or B subunits. This genetic, physical, and immunochemical evidence suggests that the E subunit is encoded in a separate LDH locus (the E locus) and is not produced by epigenetic modification of preexisting subunits encoded by either the A or B locus.

The degree of cross-reaction of one

antiserum to the LDH isozymes from different organisms often reflects the evolutionary relatedness of these organisms (*21*) and should reflect the relatedness of isozymes within a species. The A_4 and B_4 antiserums against the killifish and mackerel LDH eye isozymes (Fig. 2) reveal that some of these isozymes are related. The slight increase in mobility of the E_4 isozymes of the mackerel is not caused by the antibodies because normal serum from the same rabbit has the same effect, which is probably due to serum protein slightly displacing the E_4 isozymes. The A_4 antiserum at all concentrations reacts only with isozymes containing A subunits. The B_4 antiserum at low concentrations appreciably inactivates only those isozymes containing B subunits; however, at higher concentrations of B_4 antiserum, the E_4 isozyme and other isozymes containing E subunits are also completely inactivated. These experiments demonstrate that the B_4 and E_4 LDH isozymes are related and that the E_4 isozyme is more closely related to the B_4 than to the A_4 isozyme. This interpretation is also supported by the hierarchy of biochemical responses of $E_4 < B_4 < A_4$ observed for K_m with pyruvate as substrate, pyruvate concentration optimums, resistance to pyruvate inhibition, and susceptibility to inactivation by heat and urea (*12, 17*).

The LDH A and B loci of vertebrates appear to have evolved from a single ancestral gene, as suggested by similarity of amino acid sequence of the A and B polypeptide active sites, copolymerization of the A and B polypeptides, and the postulated linkage of the A and B loci in trout (*6, 13, 22*). Two lines of indirect evidence suggest that the LDH A locus may be more representative of the ancestral gene. First, the *A* gene is more active than the *B* gene in many vertebrate tissues, especially in flatfish, where the *B* gene function is restricted to only a few tissues (*20*). Second, the LDH A locus in teleosts appears to be less variable than the B locus, as shown by the higher frequency of electrophoretic variants at the B locus (*11, 13, 18, 23*).

In some teleosts the A and B loci have each duplicated as a result of tetraploidization, and the duplicated genes still retain a high degree of homology (*18, 24*). Although the A and B loci have probably arisen from a common ancestral gene, considerable divergence between the A and B loci has occurred and is reflected in the differences between the A_4 and B_4 isozymes in their total amino acid composition (*25*), peptide patterns (*25*), and degree of immunochemical dissimilarity (*18–20*).

The LDH *E* gene probably arose from a duplication of the *B* gene followed by mutation and divergence through selection. The *E* gene does not appear to have diverged from the *B* gene to the same extent that the A and B loci have diverged from each other. The E locus is only found in fish, which suggests that it may have arisen after fish gave rise to the amphibians. The third LDH locus in birds and mammals, the C locus, may also have arisen from the B locus, as indicated by the fact that the C locus is closely linked, perhaps contiguous, to the B locus in pigeons (*8*). However, the *C* gene product (C_4 isozyme) is not clearly related kinetically or immunochemically to either the A_4 or the B_4 isozymes (*9, 26*). An exact formulation of vertebrate LDH gene homology awaits more complete physicochemical analyses, such as peptide mapping and amino acid sequence data.

The evolution of vertebrate LDH genes may be similar to that reported for the hemoglobin system, where the γ gene arose by gene duplication from the α locus, and later the β locus arose from the γ locus (*27*). The presence in teleosts of three LDH genes coding for related polypeptides affords an excellent opportunity to examine evolutionary relationships among genes, as well as providing a basis for investigation of the specificity of gene activation during cytodifferentiation with reference to gene homology.

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Naturally Occurring Diol Lipids: Dialkoxypentanes in Porpoise (Phocoena phocoena) Jaw Oil

Abstract. *Dialkyl ethers of diols (dialkoxyalkanes), naturally occurring lipids, have been isolated from the jaw oil of the porpoise* Phocoena phocoena. *The principal constituents are dialkoxypentanes containing two 18-carbon chains. The alkoxy linkage may play an important role in the metabolism of the diol lipids.*

Bergelson and co-workers (*1*), working with both animal and vegetable sources, detected alk-1-enyl ether and acyl derivatives of ethane, propane, and butane diols in hydrolytic products of neutral lipids. However, until now, the diol lipids have not been isolated in intact form.

Although the natural occurrence of diol lipids containing an alkoxy linkage has been predicted (*2*), it has not been established. Naturally occurring alkoxy derivatives are of significance for biochemical studies of diol lipids because of metabolic relations that exist between esters and ethers of glycerol (*3*). We now describe the isolation and characterization of dialkoxypentanes from the jaw lipid of the porpoise *Phocoena phocoena*.

Porpoise jaw oil was chromato-