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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References and Notes

1. C. L. Markert and F. Møller, *Proc. Nat. Acad. Sci. U.S.* **45**, 753 (1959).
2. C. L. Markert, in *Hereditary, Developmental, and Immunologic Aspects of Kidney Disease*, J. Metcoff, Ed. (Northwestern Univ. Press, Evanston, Ill., 1962), p. 54.
3. C. Shaw and E. Barto, *Proc. Nat. Acad. Sci. U.S.* **50**, 211 (1963).
4. R. D. Cahn, N. O. Kaplan, L. Levine, E. Zwilling, *Science* **136**, 962 (1962).
5. E. Appella and C. L. Markert, *Biochem. Biophys. Res. Commun.* **6**, 171 (1961).
6. C. L. Markert, *Science* **140**, 1329 (1963).
7. A. Blanco and W. H. Zinkham, *ibid.* **139**, 601 (1963); E. Goldberg, *ibid.*, p. 602.
8. W. H. Zinkham, H. Isensee, J. H. Renwick, *ibid.* **164**, 185 (1969).
9. N. Ressler, K. L. Stitzer, D. R. Ackerman, *Biochim. Biophys. Acta* **139**, 507 (1967).
10. E. J. Massaro and C. L. Markert, *J. Exp. Zool.* **168**, 223 (1968).
11. C. L. Markert and I. Faulhaber, *ibid.* **159**, 319 (1965).
12. G. S. Whitt, *ibid.*, in press.
13. W. J. Morrison and J. E. Wright, *ibid.* **163**, 259 (1966).
14. E. Goldberg, *Science* **151**, 1091 (1966).
15. ———, J. P. Cuerrier, J. C. Ward, *Biochem. Genet.* **2**, 335 (1969).
16. H. Hitzeroth, J. Klose, S. Ohno, U. Wolf, *ibid.* **1**, 287 (1968).
17. G. S. Whitt, *Genetics* **60**, 237 (1968).
18. R. S. Holmes and C. L. Markert, *Proc. Nat. Acad. Sci. U.S.*, in press.
19. C. L. Markert and E. Appella, *Ann. N.Y. Acad. Sci.* **103**, 915 (1963).
20. C. L. Markert and R. S. Holmes, *J. Exp. Zool.*, in press.
21. A. C. Wilson, N. O. Kaplan, L. Levine, A. Pesce, M. Reichlin, W. S. Allison, *Fed. Proc.* **23**, 1258 (1964).
22. W. S. Allison, *Ann. N.Y. Acad. Sci.* **151**, 180 (1968).
23. P. H. Odense, T. M. Allen, T. C. Leung, *Can. J. Biochem.* **44**, 1319 (1966); H. O. Hodgins, W. E. Ames, F. M. Utter, *J. Fish. Res. Board Can.* **26**, 15 (1969); J. E. Wright and L. Atherton, *Genetics* **60**, 240 (1968).
24. S. Ohno and N. B. Atkin, *Chromosoma* **18**, 455 (1968); G. S. Bailey and A. C. Wilson, *J. Biol. Chem.* **243**, 5483 (1968).
25. C. L. Markert, in *Cytodifferentiation and Macromolecular Synthesis*, M. Locke, Ed. (Academic Press, New York, 1963), p. 65; A. Pesce, T. P. Fondy, F. Stolzenbach, F. Castillo, N. O. Kaplan, *J. Biol. Chem.* **242**, 2151 (1967).
26. L. J. Battellino, F. R. Jaime, A. Blanco, *J. Biol. Chem.* **243**, 5185 (1968).
27. V. M. Ingram, *Nature* **189**, 704 (1961).
28. S. H. Boyer, D. C. Fainer, E. J. Watson-Williams, *Science* **141**, 642 (1963).
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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-

graphed on thin layers of silica gel in a hexane, diethyl ether system (80 : 20, by volume) and a fraction (I) having an R_F of 0.60 was isolated. The infrared spectrum of fraction I exhibited a strong carbonyl (C=O) absorption at 1735 cm^{-1} and a strong ether (C–O) absorption at 1120 cm^{-1}. Fraction I was refluxed with lithium aluminum hydride in diethyl ether (*4*), and the reaction products were chromatographed on silica gel plates in a hexane, diethyl ether system (80 : 20, by volume). A fraction (II) having an R_F of 0.13 was isolated. The infrared spectrum of fraction II exhibited strong hydroxyl absorption at 3480 cm^{-1}, together with absorption bands characteristic of the hydrocarbon moiety. Another fraction (III) having an R_F of 0.62 was also separated (Fig. 1). The infrared spectrum of fraction III exhibited a C–O frequency characteristic of the ether linkage in addition to absorption bands associated with aliphatic C–H linkages. The nuclear magnetic resonance spectrum of fraction III revealed a triplet (near δ 3.62 ppm) which can be assigned to the protons of the methylene groups adjacent to an ether linkage. Additional signals were a multiplet peak near δ 1.80 ppm (methylene groups adjacent to double bonds) and a broad peak at δ 1.25 ppm. The latter peak can be assigned to internal methylene groups of aliphatic chains. A signal at δ 0.91 ppm was ascribed to terminal methyl groups.

The evidence thus far suggested that fraction III, which comprised 0.5 to 1.0 percent of the jaw oil, was an alkyl ether having either a glycerol or diol backbone. We found that fraction III remained unchanged after reaction with acidic ethanol (*5*). Accordingly, the possibility that fraction III contained either an acetal or a hemiacetal structure was ruled out. To establish the composition of the alkoxy chains, hydrogenated fraction III (fraction IV) was refluxed with 70 percent HI for 24 hours (*6*). Thin-layer chromatography of the product (fraction V) in pure hexane, using 1-iodooctadecane as marker, revealed the presence of 1-iodoalkanes as the principal components. Gas-liquid chromatography of the iodides (fraction V) showed 1-iodooctadecane as the major component (88 percent), together with minor amounts of shorter and longer chain iodides.

Fraction IV was treated with boron trichloride (*7*) to establish whether the backbone of fraction III was a diol or glycerol. The aqueous extract of the reaction products (fraction VI) containing the polyols was chromatographed in a chloroform, methanol system (85 : 15, by volume) with glycerol and C_2 to C_5 diols as markers. Only diols were detected after spraying the plate with ammoniacal silver nitrate (*1*). Low-temperature chromatography of the nitrate derivatives of fraction VI, with 1,5-dinitratopentane as marker (*8*), confirmed the presence of diols.

The chemical and spectral evidence showed that fraction III was a mixture of dialkoxyalkanes. Reversed-phase chromatography (*2*) of the diacetate derivatives (*1*) of fraction VI indicated that pentane diols were the main constituents. Gas-liquid chromatography (*1*) of the diacetate derivatives confirmed these results. The mass spectrum of fraction III exhibited a strong fragment peak at 102 mass units, together with peaks characteristic of various fragments of hydrocarbon chains. The peak at 102 mass units can be assigned to the fragment $C_5H_{10}O_2$ which arises from dialkoxypentanes. Thus we conclude that the jaw lipids of *Phocoena phocoena* contain dialkoxypentanes having primarily two 18-carbon chains.

Alkyl ethers of diols probably are present in a variety of natural products, together with the analogous acyl and alk-1-enyl ether structures. The roles played by the various types of diol lipids in the life processes are not known. The isolation of the dialkoxypentanes from the porpoise provides a unique opportunity for studies on the role of the alkoxy chain in the metabolism of diol lipids.

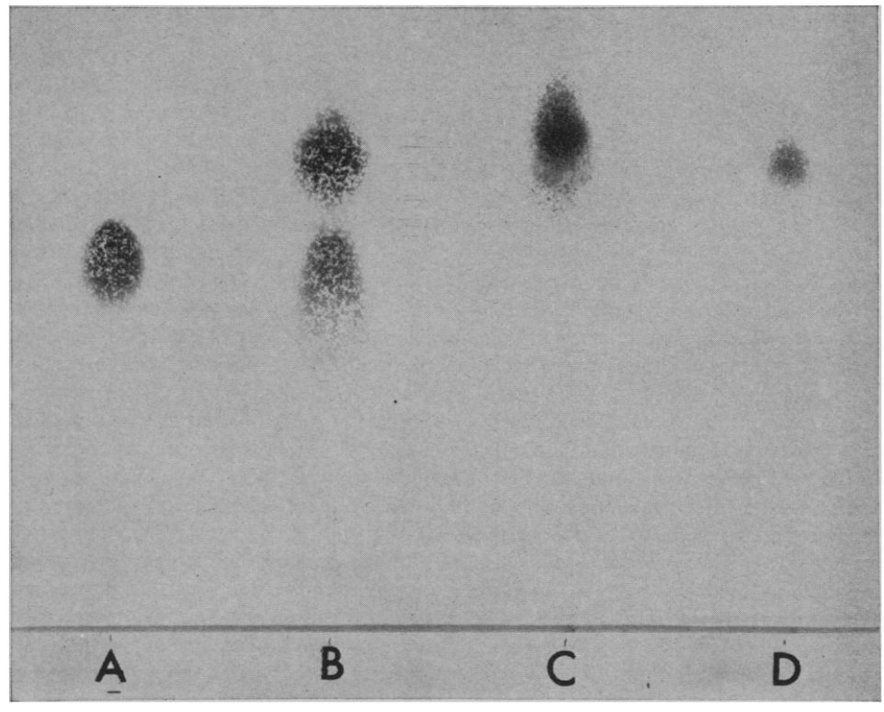

Fig. 1. Thin-layer chromatography of porpoise lipids. Adsorbent, silica gel G; solvent, hexane, diethyl ether (80:20, by volume); indicator, chromic sulfuric acid and charring. (A) pure triolein; (B) porpoise (*Phocoena phocoena*) jaw oil; (C) fraction III isolated from *Phocoena phocoena* jaw oil; (D) dihexadeclyoxyethane: $H_{33}C_{16}O—CH_2—CH_2—OC_{16}H_{33}$ (*9*).

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References and Notes

1. L. D. Bergelson, V. A. Vaver, N. V. Prokazova, A. N. Ushakov, G. A. Popkova, *Biochim. Biophys. Acta* **116**, 511 (1966).
2. W. J. Baumann, H. H. O. Schmid, H. W. Ulshöfer, H. K. Mangold, *ibid.* **144**, 355 (1967).
3. F. Snyder, in *Progress in the Chemistry of Fats and Other Lipids* (Pergamon Press, Oxford, 1969), vol. 10, part 3, p. 287.
4. W. J. Bauman, H. H. O. Schmid, J. K. G. Kramer, H. K. Mangold, *Z. Physiol. Chem.* **349**, 1677 (1968).
5. W. Bergmann and R. A. Landowne, *J. Org. Chem.* **23**, 1241 (1958).
6. K. E. Guyer, W. A. Hoffman, L. A. Horrocks, D. G. Cornwell, *J. Lipid Res.* **4**, 385 (1963).
7. M. Kates, J. S. Yengoyan, P. S. Sastry, *Biochim. Biophys. Acta* **98**, 252 (1965).
8. D. C. Malins, J. C. Wekell, C. R. Houle, *Anal. Chem.* **36**, 658 (1964).
9. Dihexadecyloxyethane provided by H. K. Mangold (The Hormel Institute, Austin, Minnesota).

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