those of the proband. Internal genitalia were those of a normal female fetus; normal fetal ovaries were present bilaterally; histological sections failed to reveal any trace of testicular tissue.

The following points are worth emphasizing. (i) Serological tests indicated absence of H-Y antigen in skin fibroblasts of the fetus and in blood leukocytes and skin fibroblasts of the proband; (ii) cytogenetic analysis failed to provide evidence for autosome-to-X translocation in any members of the family; and (iii) computer-based videodensitometric analysis of the abnormal chromosome (performed in the Department of Medical Genetics, Mayo Clinic) indicated the likelihood of a duplication of densitometric X bands p12 and 3.

The foregoing observations are consistent with the notion that testicular development is secondary to function of genes on the Y chromosome and on Xp. If X-linked regulatory genes normally served to prevent excess production of H-Y antigen, duplication of these genes might be expected to reduce production of H-Y below a certain critical threshold required for testicular differentiation. Alternatively, the extra bands might interfere with a structural element thereby thwarting production altogether (*9*). In either event the result would be subnormal expression of the testis-inducer. The XY gonad would now differentiate as an ovary; the human ovary would degenerate in the absence of the second X. We assume that the disparate gonadal histologies of proband and fetus represent different phases of the same developmental anomaly.

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References and Notes

1. K. Fredga, A. Gropp, H. Winking, F. Frank, *Nature (London)* **261**, 225 (1976).
2. S. S. Wachtel, G. C. Koo, S. Ohno, A. Gropp, V. G. Dev, R. Tantravahi, D. A. Miller, O. J. Miller, *ibid.* **264**, 638 (1976).
3. K. Fredga, A. Gropp, H. Winking, F. Frank, *Hereditas* **85**, 101 (1977). More recently these authors identified two different G-banded X chromosomes in the wood lemming, one of which is present in "sex-reversed" females. They suggest three sex chromosome constitutions in females of that species: XX, X*X, and X* Y, where X* represents a chromosome with a mutant short arm (X*p) that blocks testicular differentiation; E. W. Herbst, K. Fredga, F. Frank, H. Winking, A. Gropp, *Chromosoma* **69**, 185 (1978). Remarkably X*p is *shorter* than Xp; see text and compare with Fig. 1.
4. S. Ohno, *Sex Chromosomes and Sex-Linked Genes* (Springer-Verlag, New York, 1967).
5. R. V. Short, *Int. J. Androl. Suppl.* **2**, 21 (1978).
6. S. N. Ghosh, P. N. Shah, H. M. Gharpure, *Nature (London)* **276**, 180 (1978).
7. J. L. Simpson, *Disorders of Sexual Differentiation. Etiology and Clinical Delineation* (Academic Press, New York, 1976); J. German, J. L. Simpson, R. S. K. Chaganti, R. L. Summitt, L. B. Reid, I. R. Merkatz, *Science* **202**, 53 (1978).
8. For complete details of the case, see R. Bernstein, T. Jenkins, B. Dawson, J. Wagner, G. Dewald, G. C. Koo, S. S. Wachtel, *J. Med. Genet.*, in press.
9. It may be argued that structural testis-determinants are situated on the X chromosome and activated by genes on the Y; see, for example, J. L. Hamerton, *Nature (London)* **219**, 910 (1968); R. A. McFeely, W. C. D. Hare, J. D. Biggers, *Cytogenetics* **6**, 242 (1967). If H-Y structural genes are X-situated, then failure of H-Y synthesis in the present study could be due to a position effect resulting from duplication of a segment of Xp.
10. This work was supported in part by grants from the Rockefeller Foundation (RF-77042) and NIH (AI 11982, CA 08748, HD 00171, and HD 10065). We thank Dr. G. Dewald of the Mayo Clinic for the computer analysis.

14 June 1979

Lactate Dehydrogenases of Atlantic Hagfish: Physiological and Evolutionary Implications of a Primitive Heart Isozyme

Abstract. *Isozymes of lactate dehydrogenase from heart and muscle of Atlantic hagfish show less functional divergence than those from other fishes and higher vertebrates. The enzyme from hagfish heart (B_4) displays a higher Michaelis constant for pyruvate and lower substrate inhibition at moderate pyruvate concentrations than heart isozymes from other species. These properties support the hypothesis that the ancestral vertebrate lactate dehydrogenase was a muscle (A_4) -type enzyme and also suggest a role for the B_4 enzyme in the unusual physiology of hagfish cardiac tissue which functions under sustained hypoxic conditions.*

Most vertebrates have two predominant forms of the tetrameric enzyme lactate dehydrogenase (LDH). One of the enzymes (H_4, B_4) is the primary molecular form in heart and other aerobically respiring tissues, while the other (M_4, A_4) is the primary form in skeletal muscle tissue (*1–3*). Muscle-type isozymes of LDH retain activity in the presence of high pyruvate concentrations and have a moderately high Michaelis constant (K_m) for this substrate. This form of the enzyme functions primarily in the reduction of pyruvate to lactate under conditions of limiting oxygen supply (*1–5*). Typical heart-type isozymes of LDH are severely inhibited by pyruvate concentrations within the physiological range and display a relatively low K_m for this substrate. Substrate inhibition of the heart (B_4) isozyme is considered to promote aerobic reduction to lactate (*1–5*). The observed distribution of A_4 and B_4 isozymes in vertebrate tissues is generally consistent with this hypothesis (*3, 4*). Substantial difference in K_m for pyruvate between A_4 and B_4 isozymes within species is a characteristic also shared by widely divergent vertebrate taxa (*5, 6*).

Lactate dehydrogenases of Atlantic hagfish (*Myxine glutinosa*) provide an excellent system for examining both evolution and physiological roles of LDH isozymes. The hagfish has two forms of LDH which correspond to the A_4 and B_4 isozymes of higher vertebrates in both relative electrophoretic mobility and tissue distribution (*7, 8*). The phylogenetic position of these animals in class Agnatha (jawless fishes) places them among the most primitive living vertebrates. Among living vertebrates, hagfish are the only ones that have multiple hearts and a partially open circulatory system with sinuses reminiscent of those found in the invertebrate phyla. The two primary pumps, the branchial and portal vein hearts, are composed of true vertebrate cardiac muscle which shows a myogenic origin of rhythmic heartbeat (*9*). Like their Agnathan relatives, the lampreys (family Petromyzontidae), hagfish lack a coronary circulation for delivery of well-oxygenated blood to cardiac tissue (*10*). Both hearts pump mixed venous blood which, even when fully oxygenated, has

Table 1. Effect of preliminary incubation with the acetylpyridine analog of NAD^+ on the activity of Atlantic hagfish lactate dehydrogenases. Enzyme was first incubated for 10 minutes at 25°C in the presence of $3.3 \times 10^{-4}M$ pyruvate or $1.0 \times 10^{-2}M$ pyruvate, and $1.43 \times 10^{-6}M$ acetylpyridine analog of NAD^+. Enzyme activity of micromoles of NADH oxidized per minute was then determined in the presence of natural cofactor under conditions described (*14*). The ratio reported is the activity at $3.3 \times 10^{-4}M$ pyruvate divided by activity at $1.0 \times 10^{-2}M$ pyruvate.

Isozyme	Activity ratio
Muscle (A_4)	1.01
Heart (B_4)	2.99

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a low oxygen carrying capacity (*11*). In addition, these fish often feed by invading coelomic cavities of other dead and dying fish, and thus are exposed to extremely hypoxic conditions. These factors suggest a lack of adaptive pressure for development of highly aerobic metabolism in general, and for development of a heart-type LDH geared toward aerobic function in particular.

We have found properties of the two LDH isozymes of hagfish (*12*), which confirm that the enzyme from heart is a true B_4 isozyme, but that its function is more similar to that of the A_4 isozyme than is seen in higher vertebrates. Like other B_4 enzymes, substrate inhibition of the LDH isozyme from hagfish heart is substantially greater than for the muscle isozyme when first incubated with the acetylpyridine analog of its nicotinamide adenine dinucleotide (NAD^+) cofactor (Table 1). Sensitivity to this cofactor is an effective diagnostic tool in identifying vertebrate B_4 enzymes (*13*). Substrate inhibition of hagfish heart LDH at moderate pyruvate concentrations is lower than observed for the B_4 enzyme of other vertebrates (Table 2). The relative degree of substrate inhibition of hagfish isozymes by excess pyruvate under standard assay conditions (*14*) is, however, similar in pattern to that typically seen with A_4 and B_4 isozymes from other species (Fig. 1). Since the degree of substrate inhibition of LDH by pyruvate is considered to be of major importance in determining function of the enzyme in vivo, the hagfish B_4 isozyme appears suited for a role in anaerobic function of cardiac tissue. This suggestion is supported by studies of enzyme kinetics.

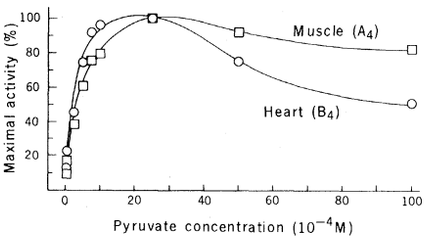

Fig. 1. The effect of pyruvate concentration on the rate of reaction of Atlantic hagfish LDH isozymes. See (*14*) for standard assay conditions.

Our estimate of the apparent K_m (*15*) for pyruvate of LDH from hagfish heart is higher than values reported for B_4 isozymes from other vertebrates and approaches values obtained for the A_4 isozyme from other species under comparable assay conditions (Table 2). The A_4 isozyme of hagfish, however, displays properties not unlike those of the muscle enzyme from other sources (Table 2).

Markert and co-workers (*7*) suggest that genes coding for A_4 and B_4 LDH's arose early in vertebrate evolution after duplication of an ancestral LDH gene. They further hypothesize that the ancestral vertebrate LDH may have been an A_4-like enzyme, since lampreys, close phylogenetic relatives of hagfish, have only the A_4 isozyme. Our results, which show less functional divergence between A_4 and B_4 isozymes in Atlantic hagfish compared to other vertebrate species, support this hypothesis. The behavior, anatomical, and physiological characteristics of hagfish further indicate that heart and other normally (in other species) oxygen-rich tissues of these animals may function under conditions of sustained hypoxia. With a low level of oxygen supply, these organs may derive energy anaerobically by partial oxidation of glucose to lactate. This would make retention of a primitive B_4 enzyme, with functional characteristics similar to the A_4 form, a distinct adaptive advantage. Metabolic studies of hagfish heart may yield additional clues to the evolution of aerobic function in vertebrate cardiac tissue.

Table 2. Michaelis constants (K_m) and substrate inhibition of lactate dehydrogenases from Atlantic hagfish and representative vertebrates. Assay conditions for all studies were as described (*14*), except as indicated.

Species	Isozyme	K_m (m*M* pyruvate)	Maximal activity at 5 m*M* pyruvate (%)	Reference
Atlantic hagfish	A_4	0.53 ± 0.04	92	*
(Myxine glutinosa)	B_4	0.45 ± 0.03	79	
Quinnat salmon	A_4	0.65 ± 0.08	89	*16*
(Oncorhynchus tshawytscha)	B'_4	0.037 ± 0.003	60	
	B_4	0.075 ± 0.003	66	
Atlantic salmon	A_4	0.40		*17*†
(Salmo salar)	B_4	0.048		
Flatfish	A_4	0.56		*18*‡
(Pseudopleuronectes americanus)	B_4	0.084		
Haddock	A_4	0.33 to 0.45		*19*§
(Melanogrammus aeglefinus)	B_4	0.037		
Mummichog	A_4	0.404 ± 0.029		*21*
(Fundulus heteroclitus)	B_4‖	0.136 to 0.159	70	*20, 21*
Chicken	A_4	0.32		*6*
(Gallus domesticus)	B_4	0.08		
Human	A_4	0.83		*22*¶
	B_4	0.08		

*This study. †0.066 m*M* NADH, 0.033*M* sodium phosphate, *p*H 7.4, 25°C. ‡Assay conditions not specified. §0.14 m*M* NADH, 0.1*M* potassium phosphate, *p*H 7.15, 25°C. ‖Range of three B_4 allozymes. ¶0.12 m*M* NADH, phosphate buffer (Sörensen), *p*H 7.4, 25°C. All other studies were performed under standard assay conditions (*14*).

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References and Notes

1. N. O. Kaplan, M. M. Ciotti, M. Hamolsky, R. E. Beiber, *Science* **131**, 392 (1960).
2. R. D. Cahn, N. O. Kaplan, L. Levine, E. Zwilling, *ibid.* **136**, 962 (1962).
3. D. M. Dawson, T. L. Goodfriend, N. O. Kaplan, *ibid.* **143**, 929 (1964).
4. J. Everse and N. O. Kaplan, in *Isozymes*, vol. 2, *Physiological Function*, C. L. Markert, Ed. (Academic Press, New York, 1975), p. 29.
5. C. J. Masters and R. S. Holmes, in *Advances in Comparative Physiology and Biochemistry*, O. Lowenstein, Ed. (Academic Press, New York, 1974), vol. 5, p. 110.
6. A. Pesce *et al.*, *J. Biol. Chem.* **242**, 2151 (1967).
7. C. L. Markert, J. B. Shaklee, G. S. Whitt, *Science* **189**, 102 (1975).
8. H. A. Dahl and H. Korneliussen, *Comp. Biochem. Physiol.* **55B**, 381 (1976).
9. K. Johansen, in *The Biology of Myxine*, A. Brodal and R. Fange, Eds. (Universitetsforlaget, Oslo, 1963), p. 289.
10. G. Bloom, E. Ostlund, R. Fange, in *ibid.*, p. 317.
11. C. Manwell, *Biol. Bull.* **115**, 227 (1958).
12. Hagfish LDH's were isolated from white skeletal muscle and portal and branchial hearts by affinity chromatography of tissue extracts on blue dextran-agarose columns. Cofactor elution of the enzyme was used and residual reduced nicotinamide adenine dinucleotide (NADH) was removed by extensive dialysis overnight at 4°C. An approximately 300-fold purification was realized for each isozyme.
13. N. O. Kaplan, J. Everse, J. Admiraal, *Ann. N.Y. Acad. Sci.* **151**, 400 (1968).
14. Standard assay conditions for determination of LDH activity were: 0.15 m*M* NADH, 0.1*M* potassium phosphate, *p*H 7.5, at 25°C. Pyruvate concentration was varied as required.
15. Apparent K_m's were estimated as described [G. N. Wilkinson, *Biochem. J.* **80**, 324 (1961)].
16. S. T. Lim, R. M. Kay, G. S. Bailey, *J. Biol. Chem.* **250**, 1790 (1975).
17. H. Gesser and L. E. Sundell, *Int. J. Biochem.* **2**, 462 (1971).
18. C. L. Markert and R. S. Holmes, *J. Exp. Zool.* **171**, 85 (1969).
19. G. F. Sensabaugh and N. O. Kaplan, *J. Biol. Chem.* **247**, 585 (1972).
20. A. R. Place and D. A. Powers, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 2354 (1979).
21. A. R. Place, personal communication.
22. J. H. Wilkinson and W. A. Withycombe, *Biochem. J.* **97**, 663 (1965).

23. We thank Drs. J. D. McCleave, G. N. Somero, and G. S. Whitt for reading the manuscript. Hagfish were supplied by Dr. Richard Peterson, Fisheries and Oceans, St. Andrews, New Brunswick, Canada. Supported in part by NSF grant SER 77-06950 to the Migratory Fish Research Institute. Equipment used was purchased by a University of Maine faculty research equipment award and by NSF grant PCM 77-16838 to B.D.S.

30 August 1979; revised 5 November 1979