blocks an octopamine receptor in the locust (*25*), stimulated egg laying in *C. elegans* hermaphrodites not exposed to any other drugs: ten adult hermaphrodites placed in microtiter wells containing phentolamine (10 mg/ml) in M9 buffer (*10*) released 53 eggs in 30 minutes, whereas untreated control animals released no eggs. This observation suggests that endogenous octopamine also inhibits egg laying. The increased levels of endogenous octopamine seen in adults may reflect the fact that egg laying is an adult behavior.

The finding that serotonin and octopamine affect pharyngeal pumping and egg laying oppositely suggests that these amines function as antagonists physiologically. Consistent with this hypothesis, octopamine depressed serotonin-stimulated egg laying (Fig. 2a). (Octopamine did not, however, prevent serotonin-stimulated pharyngeal pumping.) Antagonistic effects of these amines have been observed in other invertebrates (*25, 26*).

We have identified mutant strains of *C. elegans* with reduced levels of serotonin or octopamine. The *unc-86* mutants (*9*) are deficient in yellow FIF of the NSM's. Three mutants with well-characterized neuronal defects—*daf-10(e1387)* (*27*), *che-3(e1124)* (*28*), and *osm-3(p802)* (*29, 30*)—lack detectable levels of octopamine (that is, contain less than 0.02 μg/g). We hope that these and additional mutants may be used to define the roles of serotonin and octopamine in *C. elegans*.

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Physiological Basis for Swimming Endurance Differences Between LDH-B Genotypes of *Fundulus heteroclitus*

Abstract. *Adenosine triphosphate levels in erythrocytes are correlated with LDH-B genotype in* Fundulus heteroclitus. *Adenosine triphosphate is the fish's allosteric modifier of hemoglobin oxygen affinity. Since oxygen delivery to muscle affects swimming performance, fish of each homozygous LDH-B phenotype were swum to exhaustion at 10° or 25°C to determine whether in vitro differences attributed to the LDH-B allelic isozymes were manifest in vivo. At 10°C, the critical swimming speed of the LDH-B^{a}B^a phenotype was 3.6 body lengths per second, whereas that of the LDH-B^{b}B^b phenotype was 4.3 body lengths per second. At 25°C there were no differences between LDH-B phenotypes in erythrocyte adenosine triphosphate levels, blood oxygen affinity, or swimming performance.*

Adenosine triphosphate (ATP) is the major organic phosphate in the erythrocytes of many fish species (*1*). Its role as an allosteric modifier of fish hemoglobins is similar to that of 2,3-diphosphoglycerate in mammalian red blood cells in that it decreases the affinity of hemoglobin for oxygen, facilitating oxygen delivery to tissues (*2*).

Erythrocyte organic phosphate concentrations are heritable traits in humans (*3*), rats (*4*), and fish (*5*). In humans, this trait is correlated with genetic variability of both pyruvate kinase and hexokinase (*3*). In the fish *Fundulus heteroclitus*, erythrocyte organic phosphate (that is, ATP) levels are correlated with genetic variation at the LDH-B locus (*5*). Erythrocyte ATP levels are lower in fish with the LDH-B^{a}B^a phenotype than they are in those with the LDH-B^{b}B^b phenotype, and concentrations in heterozygotes (LDH-B^{a}B^b) are intermediate (*5*). Since ATP is the major organic phosphate in *Fundulus* red cells (*1, 6, 7*), oxygen affinity differences also exist between LDH-B phenotypes (*5*). However, the mechanism by which genetic variation at the LDH-B locus affects erythrocyte ATP levels is not known. Either the LDH-B isozyme influences erythrocyte ATP metabolism, or the LDH-B locus is linked to a second locus that affects ATP production.

Kinetic analyses of purified LDH-B allelic isozymes indicated that the greatest catalytic differences between LDH-B^{a}B^a and LDH-B^{b}B^b exist at low temperature (10°C), with no significant difference at high temperature (approximately 25°C) (*8*). Thus, if the LDH-B isozyme has a metabolic influence on erythrocyte ATP concentration, differences in ATP and blood oxygen affinity should exist at acclimation temperatures below 25°C. Furthermore, since organic phosphate amplifies the Bohr effect of *F. heteroclitus* hemoglobins (*6, 7*), this phenomenon should be exaggerated at low blood *p*H values, like those produced during swimming performance experiments. We now report that swimming performance is highly correlated with genetic variation at the LDH-B locus for

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Fundulus acclimated to 10°C, whereas no such differences exist for fish acclimated to 25°C. These results provide insight into the evolutionary mechanisms through which genetic variation at the molecular level is reflected in the whole animal.

Fish were obtained from the western shore of Delaware Bay, typed for LDH-B phenotype, and acclimated for 1 month in 300-gallon circular tanks at 10° or 25°C and salinity of 10 ppt (*9*). After the acclimation period, analyses were made of 12 "resting" fish randomly selected from each of the two homozygous LDH-B phenotypes, and the remaining fish were swum to exhaustion in a closed water tunnel at the acclimation temperature (*10*). The exhausted fish were killed immediately, and the appropriate biochemical and physiological analyses were made (*11*).

Among resting fish acclimated to 10°C, hematocrit, blood *p*H, blood oxygen affinity, serum lactate, liver lactate, and muscle lactate were not significantly different between the two LDH-B homozygous phenotypes (Table 1). When fish acclimated to 10°C were exercised to the point of fatigue, a significant change occurred in all of these measures. The data in Table 1 show that the LDH-B^bB^b phenotype was able to sustain a swimming speed 20 percent higher than that of the LDH-B^aB^a phenotype. Blood oxygen affinity, serum lactate, and muscle lactate also differed between the two phenotypes. Since the rate of lactate accumulation was the same for the LDH-B phenotypes (*12*), LDH-B^bB^b fish accumulated more lactate in the blood and muscle simply because they swam longer.

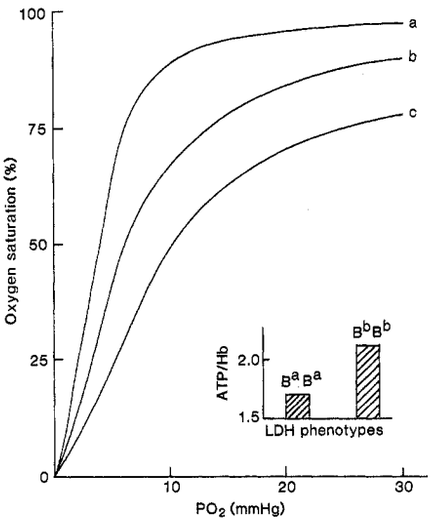

Fig. 1. Oxygen equilibrium curves for whole blood of *Fundulus heteroclitus* acclimated to 10°C, as determined with an oxygen dissociation analyzer (Aminco). The ordinate is the percentage saturation of hemoglobin by oxygen, and the abscissa is the partial pressure of oxygen (PO_2). Oxygen equilibrium curves of blood from (a) resting fish of both LDH-B phenotypes, (b) LDH-B^aB^a swum to exhaustion, and (c) LDH-B^bB^b swum to exhaustion. The intraerythrocyte ratio of ATP to hemoglobin (*ATP/Hb*) in resting fish is 1.65 ± 0.12 for LDH-B^aB^a and 2.11 ± 0.22 for LDH-B^bB^b.

In an extensive analysis of the binding of ATP to deoxyhemoglobin in carp, we showed that the organophosphate-hemoglobin affinity constants change by two orders of magnitude between *p*H 8 and *p*H 7 (*13*). The same general phenomenon appears to be true for *F. heteroclitus* hemoglobin (*2, 14*). In resting *Fundulus* at 10°C, the blood *p*H was about 7.9 (Table 1). At this *p*H, the difference in erythrocyte ATP between LDH-B phenotypes—the ratio of ATP to hemoglobin was 1.65 ± 0.12 for LDH-B^aB^a and 2.11 ± 0.22 for LDH-B^bB^b—is not reflected as a significant difference in blood-oxygen affinity. However, as blood *p*H falls with increasing exercise, the organophosphate-hemoglobin affinity constant increases, and differences in oxygen affinity between homozygous LDH-B genotypes become apparent (Fig. 1). As blood *p*H is lowered, ATP amplifies the dissociation of oxygen from *F. heteroclitus* hemoglobin; the more ATP, the greater the effect (*6, 7*). This difference is translated into a differential ability to deliver oxygen to muscle tissue, which in turn affects swimming performance. The LDH-B^bB^b phenotype can deliver between 18 and 40 percent more oxygen to muscle tissue than the LDH-B^aB^a phenotype at maximum swimming speed (*15*).

In fish acclimated to 25°C, there were no significant differences in erythrocyte ATP concentrations. The ratio of ATP to hemoglobin was 1.45 ± 0.24 for LDH-B^aB^a and 1.65 ± 0.31 for LDH-B^bB^b. In addition, there were no significant differences between LDH-B phenotypes in any of the other parameters (Table 1). Since there were differences at 10°C, but none at 25°C, the data are consistent with the hypothesis that the LDH-B isozyme influences red cell ATP levels. Additional tests of this hypothesis will include studies of (i) the role of LDH-B in the regulation of metabolic flux, (ii) the partitioning of carbon within erythrocytes, and (iii) genetic analyses of loci linked to LDH-B.

Fundulus heteroclitus is found in large populations along the Atlantic Coast from the cold waters of Newfoundland to

Table 1. Response to swimming stress by *Fundulus heteroclitus* LDH-B phenotypes acclimated to 10° and 25°C. Values are means ± standard errors of the mean for 10 to 12 measurements. P_{50} is the partial pressure of oxygen at which hemoglobin is half saturated; hemoglobin-oxygen affinity studies were done at 21.5°C. N.S., not significant.

Parameter	10°C			25°C		
	LDH-B^aB^a	LDH-B^bB^b	*P*	LDH-B^aB^a	LDH-B^bB^b	*P*
		Resting fish				
Hematocrit	23 ± 1	24 ± 1	N.S.	24 ± 1	25 ± 2	N.S.
Blood *p*H	7.87 ± 0.05	7.84 ± 0.04	N.S.	7.40 ± 0.05	7.48 ± 0.04	N.S.
P_{50} (mmHg)	4.2 ± 0.2	3.8 ± 0.2	N.S.	5.0 ± 0.3	4.7 ± 0.2	N.S.
Serum lactate (m*M*)	1.82 ± 0.28	1.37 ± 0.15	N.S.	2.6 ± 0.69	4.5 ± 0.86	N.S.
Liver lactate (μmole/g)	0.390 ± 0.055	0.383 ± 0.044	N.S.	1.8 ± 0.42	1.53 ± 0.39	N.S.
Muscle lactate (μmole/g)	7.93 ± 0.75	8.17 ± 1.02	N.S.	11.8 ± 2.2	12.5 ± 1.6	N.S.
		Exercised fish				
Critical swim speed (body lengths per second)	3.6 ± 0.12	4.3 ± 0.1	*	5.6 ± 0.3	5.8 ± 0.3	N.S.
Hematocrit	30 ± 3	35 ± 2	N.S.	36 ± 1	38 ± 2	N.S.
Blood *p*H	7.24 ± 0.04	7.15 ± 0.05	N.S.	7.12 ± 0.03	7.09 ± 0.09	N.S.
P_{50} (mmHg)	6.57 ± 0.5	9.1 ± 0.5	*	7.4 ± 0.6	7.2 ± 0.5	N.S.
Serum lactate (m*M*)	12.19 ± 1.21	16.29 ± 0.79	*	23.4 ± 2.5	17.5 ± 1.6	N.S.
Liver lactate (μmole/g)	1.39 ± 0.12	1.56 ± 0.17	N.S.	6.6 ± 1.2	5.0 ± 0.6	N.S.
Muscle lactate (μmole/g)	17.08 ± 1.86	24.01 ± 1.46	*	23.2 ± 4.8	20.7 ± 1.6	N.S.

*Significant at $P < .05$.

the semitropical estuaries of upper Florida (*16*). Evidence has been presented that the gene frequencies for a number of loci change dramatically along the animal's distribution (*17*). Since this species has a restricted home range of approximately 36 m (*18*), the gene frequencies of specific populations could reflect adaptations to local environmental conditions (*17*). The LDH-B locus is particularly noteworthy because populations from the cold waters of Maine are essentially fixed for the LDH-B^bB^b genotype, whereas southern populations are fixed for the LDH-B^aB^a genotype (*17*). Since the catalytic efficiency of the purified allelic isozymes is consistent with the "cline" in LDH-B gene frequency, a selective scenario has been proposed (*8*). Our recent studies on the role of the LDH-B isozymes in the development and hatching of *Fundulus* embryos (*18*) and the swimming performance results reported herein add confidence to that hypothesis.

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10. The stamina tunnel is described by S. I. Hartwell and R. C. Otto [*Trans. Am. Fish. Soc.* **107**, 793 (1978)]. Each fish was acclimated in the tunnel for 1 hour at a water velocity of 14.1 cm/sec. Velocity was increased by 7.3 cm/sec in 10-minute intervals until the fish became fatigued. The critical swimming speed (U_{crit}, expressed in body lengths per second), which is the theoretical speed at which a fish can swim indefinitely, was calculated by the method of J. R. Brett [*J. Fish. Res. Board Can.* **21**, 1183 (1964)] as follows:
$$U_{crit} = [A + 7.3\ (t_{(out)}/10)]/l$$
where A is the water velocity during the last time interval successfully completed, $t_{(out)}$ is the time (in minutes) spent at the highest water velocity obtained, and l is the total body length (in centimeters).
11. Liver and muscle samples were frozen immediately on a tissue press immersed in liquid nitrogen. Blood was drawn from the caudal artery into two heparinized microhematocrit tubes. The blood from one tube was centrifuged for 3 minutes, the hematocrit was determined, and the serum was frozen in liquid nitrogen. The blood from the other tube was used to measure *p*H and blood-oxygen affinity (*7*). Frozen liver or muscle samples were ground to a fine powder in a mortar at liquid nitrogen temperature, extracted with 1*M* $HClO_4$, sonicated, and centrifuged to remove protein. Liver extracts were filtered through a Fluorisil column to remove endogenous fluorescence. One milliliter of each extract was titrated to *p*H 6.0 with 2*M* $KHCO_3$ and centrifuged, and the supernatant was assayed for lactate. Serum samples were assayed directly for lactate [J. V. Passoneau, in *Methods of Enzymatic Analysis*, H. V. Bergmeyer, Ed. (Academic Press, New York, 1974), pp. 1458–1470].
12. In both phenotypes, serum lactate concentration (SL) increased as a function of critical swimming speed (U_{crit}) according to the relation $SL = 5.84\ (U_{crit}) - 8.82$. Muscle lactate concentration (ML) increased as a function of U_{crit} in the LDH-B^aB^a phenotype according to the relation $ML = 6.67\ (U_{crit}) - 4.68$. The corresponding function for the LDH-B^bB^b phenotype was $ML = 5.83\ (U_{crit}) - 4.00$. There is no significant difference between the two phenotypes in the slopes of the two lines. Thus, both phenotypes accumulate lactate at the same rate.
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Crystallization of the Tetramer of Histones H3 and H4

Abstract. *Crystals of the histone tetramer $(H3\text{-}H4)_2$ from calf thymus have been grown. The crystals yield x-ray diffraction patterns with Bragg spacings as small as 3.5 angstroms. Crystals grown from two types of preparations have the symmetry of the space group $P6_1$ (or $P6_5$). The best crystals were grown from histones that had the amino terminal arms removed by mild trypsinization.*

The eukaryotic mitotic chromosome is a highly compact structure containing a DNA molecule which, if unfolded, would have a contour length 1 million times longer than an average chromosome. At least the first of several successive levels of the DNA compaction process results from supercoiling of the DNA around an octameric core of the inner histones (*1*). The supercoiling property is inherent in the DNA double helix (*2*), and in eukaryotes it is expressed as a left-handed supercoil when the helix associates with the octamer of the inner histones. The stoichiometry of the octamer is $(H2A, H2B, H3, H4)_2$. Successive DNA-histone complexes along a DNA backbone result in a chromatin fiber with a diameter of approximately 100 Å, which upon electron microscopy exhibits a characteristic beads-on-a-string appearance and in which the DNA is compacted linearly six- to sevenfold. Brief digestion of chromatin with micrococcal nuclease severs the DNA in the spacer region and releases the repeating structural units, called nucleosomes, each containing about 200 base pairs of DNA, the inner histone octamer, and histone H1. A review of nucleosome structure can be found in (*1*).

Until now, the only crystallographic data on the structure of the nucleosome originated from a low-resolution study (*3*). Crystals of nucleosome core particles have been analyzed to a resolution of 25 Å by electron microscopy and x-ray and neutron diffraction; ordered precipitates of histone octamer have been analyzed by electron microscopy. From this has emerged a picture of the nucleosome core particle as a disklike object 110 Å in diameter and 55 Å thick. One and three-quarters turns of a DNA duplex (146 base pairs) are wound around the histone octamer in a superhelix of pitch 28 Å.

The inner histones form the octamer by the assembly of two H2A-H2B dimers with one $(H3\text{-}H4)_2$ tetramer (*4*). Kornberg and Thomas (*5*) observed that the histones H3 and H4 interact to form a tetramer. Work by others (*6, 7*) substantiated this observation and demonstrated that the tetramer is stable over a wide range of ionic strengths.

A wealth of experimental data exists on the reconstitution of nucleosomes and chromatin from DNA and histones. The $(H3\text{-}H4)_2$ tetramer is the central component organizing DNA into nucleosomes. When a complex of H3, H4, and DNA is digested with micrococcal nuclease, it exhibits a nuclease sensitivity pattern similar to that of chromatin (*8*). A study of histone distribution during chromosome replication emphasizes the central role of the $(H3\text{-}H4)_2$ tetramer by demon-

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