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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References and Notes

1. R. G. Gillen and A. Riggs, *Comp. Biochem. Physiol.* **38B**, 585 (1971); S. C. Wood and K. Johanson, *Nature (London) New Biol.* **237**, 278 (1972); M. A. Granet, M. B. Weiss, D. A. Powers, *Biol. Bull. (Woods Hole, Mass.)* **145**, 437 (1973); D. A. Powers, *Ann. N.Y. Acad. Sci.* **241**, 472 (1974).
2. Reviewed in D. A. Powers, *Am. Zool.* **20**, 139 (1980).
3. G. J. Brewer, *Biochem. Genet.* **1**, 25 (1967); ———, F. J. Oelshlegel, J. W. Eaton, *Ann. N.Y. Acad. Sci.* **241**, 513 (1974).
4. N. A. Nobel, G. J. Brewer, F. J. Oelshlegel, *Biochem. Genet.* **16**, 39 (1978).
5. D. A. Powers, G. S. Greaney, A. R. Place, *Nature (London)* **277**, 240 (1979).
6. P. A. Mied and D. A. Powers, *J. Biol. Chem.* **253**, 3521 (1978).
7. D. A. Powers *et al.*, *Comp. Biochem. Physiol.* **62A**, 67 (1979).
8. A. R. Place and D. A. Powers, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 3254 (1979).
9. Fish were typed by the method of A. R. Place and D. A. Powers [*Biochem. Genet.* **16**, 577 (1978)]. The average total length of the fish was 8.2 ± 0.1 (S.E.) cm. All fish were sexually regressed throughout the experiment and equal numbers of males and females were sampled.
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.
13. G. S. Greaney, M. K. Hobish, D. A. Powers, *J. Biol. Chem.* **255**, 445 (1980).
14. G. S. Greaney and D. A. Powers, unpublished observations.
15. This estimate was made from the binding isotherms in Fig. 1, assuming a tissue PO_2 between 15 and 30 mmHg; this is a conservative estimate because it employs values determined for mixed venous blood [J. W. Kiceniuk and D. R. Jones, *J. Exp. Biol.* **69**, 247 (1977)]. Muscle PO_2 must be substantially less than the PO_2 of venous blood. However, during our experiments, the arterial blood of *Fundulus* should have been saturated with oxygen because the PO_2 of the water was greater than 150 mmHg.
16. V. A. Lotrich, *Ecology* **56**, 191 (1974).
17. D. A. Powers and D. W. Powers, in *The Isozymes*, C Markert, Ed. (Academic Press, New York, 1975), vol. 4, pp. 63–84; R. E. Cashon, R. J. Van Beneden, D. A. Powers, *Biochem. Genet.* **19**, 715 (1981); J. B. Mitton and R. K. Koehn, *Genetics* **79**, 97 (1975); D. A. Powers and A. R. Place, *Biochem. Genet.* **16**, 593 (1978).
18. L. DiMichele and D. A. Powers, *Nature (London)* **296**, 563 (1982).
19. We thank J. Mitton and R. Koehn for reading the manuscript, K. Kelly and L. Linker for technical assistance, D. Powers for artistic services, and D. Warr for secretarial services. Supported by NSF grant DEB 79-2216 (to D.A.P.) and NIH postdoctoral fellowship 1-F32-GMO7889-01 (to L.D.M.). This is contribution 1147 from the Department of Biology, Johns Hopkins University, Baltimore, Md. 21218.

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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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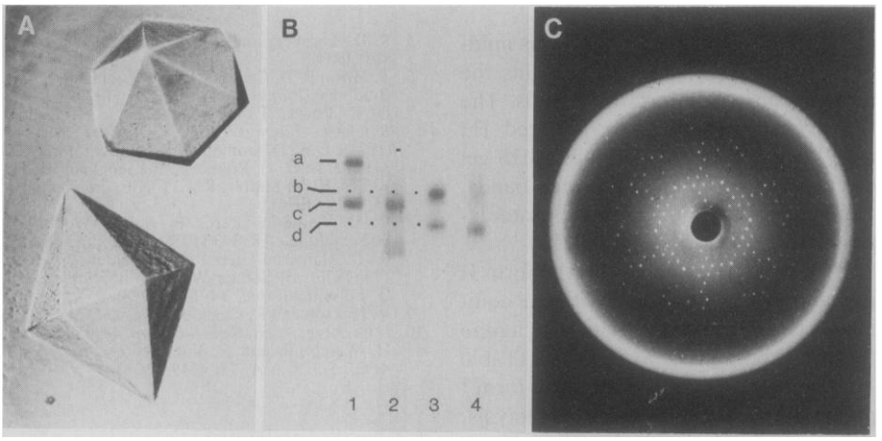

Fig. 1. The form I crystals were grown using the hanging drop version of the standard vapor diffusion technique (*15*). Calf thymus tetramer (5 to 15 mg/ml) prepared as described in (*4*) was dialyzed into 0.2*M* NaCl, 0.25 m*M* EDTA, buffered to *p*H 7.5 with tris-HCl. This solution was mixed with a solution of 6 to 12 percent (weight to volume) 6000-dalton PEG, 0.2*M* NaCl, and 0.25 m*M* EDTA, buffered with NaH_2PO_4 and equilibrated with 1 ml of the same PEG buffer. Crystals grew in 2 to 3 months and were often small. (A) The form II crystals are hexagonal bipyramids 0.2 mm long. The clipped calf thymus tetramer was prepared from trypsinized octamer (*11*). The crystallization method was the same as for form I, and seeding was performed as described in the text. (B) Acid-urea gel electrophoresis was carried out as described in (*16*). (Lane 1) Five micrograms of calf thymus tetramer (*a*, H3; *b*, H4). (Lane 2) The protein from a few form I crystals. (Lane 3). The protein from the trypsinized tetramer (*c*, trypsinized H3; *d*, trypsinized H4), the starting material for form II crystals. (Lane 4) The protein from a few form II crystals. See text for details. (C) A 10° screened precession photograph of the *hk*0 reflections from a form II crystal taken with a standard x-ray generator and a collimated beam. Sixfold symmetry is apparent.

strating that it is the first histone subunit to complex with newly replicated DNA, the H2A-H2B dimer subunit being added only after this complex is formed (*9*).

We began experiments to crystallize the H2A-H2B dimer, the $(H3\text{-}H4)_2$ tetramer, and the octamer because we believe that knowing the precise structures of subassemblies that occur in solution is important for understanding the regulation of their assembly into the nucleosome. In addition, crystals giving high-resolution diffraction information could improve the interpretation of the intact nucleosome structure.

In our attempts to crystallize the $(H3\text{-}H4)_2$ tetramer, we considered that the conformational flexibility of the amino-terminal arms (*6*) or their microheterogeneity arising from postsynthetic modification (*10*) might be interfering with crystallization. We therefore attempted to crystallize a preparation of $(H3\text{-}H4)_2$ tetramer from which the flexible arms had been removed (clipped tetramer) by controlled mild trypsinization (*11*). This is part of a broader study aimed at determining the role of amino-terminal and carboxyl-terminal arms of histones in the structure of chromatin. A detailed study of the properties of mildly trypsinized histones has recently been completed (*12*).

We have grown two crystal forms (I and II) of the $(H3\text{-}H4)_2$ tetramer from calf thymus that are suitable for x-ray diffraction analysis. Form I crystals (native tetramer starting material) took 3 months to grow and are rarely larger than 0.250 mm by 0.08 mm. The material in these crystals has apparently been proteolyzed during growth (see below). Crystals of form II (Fig. 1A, clipped tetramer) were grown under the conditions described in the legend to Fig. 1. Crystals appeared in a few days in the hanging drops equilibrated with 6 percent polyethylene glycol (PEG). Equilibrating the crystals grown from 6 percent PEG with a higher concentration of PEG causes the crystals to grow larger. Seeding those crystals into hanging drops with fresh protein and equilibrating the hanging drop first with 6 percent and later with 12 percent PEG causes the crystals to continue to increase in size. We now have crystals more than 0.4 mm wide. Forms I and II appear to be isomorphous, although they have different habits. The relatively isometric hexagonal bipyramids in form II are more convenient for x-ray study. In addition they grow more quickly and to larger size. The crystals have the symmetry of the space group $P6_1$ or $P6_5$, with $a = b = 81$ Å and $c = 100$ Å. At low resolution, however, approximate twofold rotational symmetry is apparent normal to the hexagonal axis, giving rise to the approximate space group $P6_122$ or $P6_522$. In the latter space group there are 12 asymmetric units per unit cell. From the known molecular mass of the clipped tetramer (43,000 daltons), and its approximate density (1.35 g/cm^3), it can be shown that 12 half-tetramers would occupy about 56 percent of the volume of the unit cell. Fewer than six tetramers cannot provide 12 asymmetric units; a multiple of 6 will not fit into the unit cell. Thus, if the space group were $P6_122$, there would be half a tetramer in the crystallographic asymmetric unit. For the correct space group, $P6_1$, there is a whole tetramer in the asymmetric unit, and the tetramer has an approximate noncrystallographic twofold axis in it.

Figure 1B shows a number of acid-urea (Chalkley) gels illustrating the state of the protein in the crystals. Lane 1 shows standard calf thymus tetramer. Lane 2 contains form I crystals and shows bands for H4 and for partially proteolyzed H3. The same protein sample gave rise to a single band on sodium dodecyl sulfate (SDS)–polyacrylamide gel electrophoresis (not shown), indicating that the proteolyzed form of H3 was migrating with the intact H4 in this system. Lane 3 contains clipped tetramer

Table 1. Amino acid analysis of form I and form II preparations. All data were normalized to valine = 14 residues per one clipped H3 plus H4 unit. The "calculated" values are based on clipped H3 and clipped H4 fragments considered to be the most likely products of limited trypsinization, that is, comprising residues 38 to 135 and 21 to 102, respectively.

Amino acid	Analysis		
	Form I	Form II	Calculated
Cysteine	1.4	4.2	2
Aspartic acid/ asparagine	10.8	11.2	10
Threonine	12.4	10.2	12
Serine	5.2	5.1	4
Glutamic acid/ glutamine	20.8	21.5	19
Proline	2.0	1.4	5
Glycine	10.8	12.3	12
Alanine	15.5	16.3	16
Valine	14.0	14.0	14
Methionine	0.4	1.9	3
Isoleucine	8.3	8.2	13
Leucine	20.0	19.5	18
Tyrosine	3.8	4.7	7
Phenylalanine	7.3	8.5	6
Histidine	2.3	3.0	3
Lysine	11.7	12.4	11
Arginine*	15.7	15.0	25
Total	161.7	169.4	180

*The amount of arginine detected in the control preparation of intact tetramer was similarly lower than expected (data not shown). Indeed, correction of the totals of form II for the difference in arginine between calculated and observed brings the total number of residues to 180 again.

prepared from octamer, which was mildly trypsinized (*11*). Lane 4 contains the protein from a few form II crystals. The bands characteristic of trypsinized H3 and H4 are apparent. Since SDS-gel electrophoresis yielded three bands, there might be some additional proteolysis in the crystal (not shown).

The amino acid analysis of the form II preparation is consistent with an equimolar H3-H4 complex with the amino termini of both proteins removed (Table 1). The amino acid analysis of the form I preparation indicates similar proteolysis.

Figure 1C shows a 10° screened precession photograph of a form II crystal about 0.4 mm across. This film shows reflections at 4.4-Å resolution, the limit set by the precession angle. Small-angle, unscreened photographs show reflections past 3.5-Å resolution that should be measurable. The crystals are surprisingly resistant to radiation damage. Form I crystals photographed at the Cornell High Energy Synchrotron Source (CHESS) with an extremely intense, focused monochromatic beam gave films comparable to that shown in Fig. 1C.

A number of studies demonstrate that chromatin briefly digested with trypsin has characteristics similar to that of undigested chromatin. Trypsinized chromatin yields 145–base pair repeats of DNA when treated with micrococcal nuclease. Trypsinized nucleosomes are stable particles that preserve the sensitivity of the nucleosomal DNA at ten base intervals to deoxyribonuclease I digestion (*6*, *7*) and that retain a buried sulfhydryl group (*13*). Trypsinized histones can be reconstituted with DNA into particles resembling nucleosomes (*14*). Therefore we believe that the structure of the tetramer with the amino termini removed is physiologically relevant.

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References and Notes

1. J. D. McGhee and G. Felsenfeld, *Annu. Rev. Biochem.* **49**, 1115 (1980).
2. T. H. Eickbush and E. N. Moudrianakis, *Cell* **13**, 295 (1978).
3. J. T. Finch, L. C. Lutter, D. Rhodes, R. S. Brown, B. Rushton, M. Levitt, A. Klug, *Nature (London)* **269**, 29 (1977); A. Klug *et al.*, *ibid.* **287**, 509 (1980).
4. T. H. Eickbush and E. N. Moudrianakis, *Biochemistry* **17**, 4955 (1978); J. E. Godfrey, T. H. Eickbush, E. N. Moudrianakis, *ibid.* **19**, 1339 (1980).
5. R. D. Kornberg and J. O. Thomas, *Science* **184**, 865 (1974).
6. T. Moss, P. D. Cary, C. Crane-Robinson, E. M. Bradbury, *Biochemistry* **15**, 2261 (1976).
7. D. E. Roark, T. E. Geoghian, G. H. Keller, *Biochem. Biophys. Res. Commun.* **59**, 542 (1974); J. A. D'Amma and I. Isenberg, *ibid.* **61**, 343 (1974); D. E. Roark, T. E. Geoghian, G. H. Keller, K. V. Matter, R. L. Engle, *Biochemistry* **15**, 3019 (1976).
8. R. D. Camerini-Otero, B. Sollner-Webb, G. Felsenfeld, *Cell* **8**, 333 (1976); B. Sollner-Webb, R. D. Camerini-Otero, G. Felsenfeld, *ibid.* **9**, 179 (1976); B. Sollner-Webb, W. Melchior, Jr., G. Felsenfeld, *ibid.* **14**, 611 (1978).
9. V. Jackson and R. Chalkley, *ibid.* **23**, 121 (1981).
10. I. Isenberg, *Annu. Rev. Biochem.* **48**, 159 (1979).
11. H. Weintraub and F. Van Lente, *Proc. Natl. Acad. Sci. U.S.A.* **71**, 4249 (1974).
12. C. Hatch and E. N. Moudrianakis, in preparation.
13. N. T. N. Wong and E. P. M. Candido, *J. Biol. Chem.* **253**, 8263 (1978).
14. J. P. Whitlock, Jr., and R. T. Simpson, *ibid.* **252**, 6516 (1977); J. P. Whitlock, Jr., and A. Stein, *ibid.* **253**, 3857 (1978).
15. A. McPherson, Jr., *Methods Biochem. Anal.* **23**, 233 (1976).
16. S. Panyim and R. Chalkley, *Arch. Biochem. Biophys.* **130**, 337 (1969).
17. Supported by a grant from the American Cancer Society (E.N.M.) and an internal grant from the Johns Hopkins University School of Medicine (E.L.). We thank the Cornell High Energy Synchrotron Source for the use of its facilities and D. Powers for the amino acid analysis.

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Epidermis of Human Skin: Pyroelectric and Piezoelectric Sensor Layer

Abstract. *The epidermis of live human skin has a permanent electric dipole moment perpendicular to its surface. Voltage responses to a rapid change of temperature are pyroelectric, while voltage responses to pressure pulses are piezoelectric in nature. The time course of the responses depends on* dX/dt (X, *temperature or pressure). The epidermal surface can react to all physical environmental influences to which nonbiological pyroelectric materials are known to respond. Epidermal voltage signals can be perceived through the intraepidermal and the superficial dermal nervous network. The pyroelectric and piezoelectric properties are also measurable on dead, dry skin samples.*

We examined the hair-free surface of the backs of the fingers of ten test persons. A measuring electrode and a reference electrode were applied to the skin surface. The electrodes (area, 10 mm^2) were placed approximately 1 cm apart; they consisted of 5 μl of colloidal graphite (Aquadag) held in place with adhesive rings (*1*). The measurements were carried out in a Faraday cage. Additional measurements were made on skin preparations examined within 1 to 2 hours after surgery. The specimens (area, ~ 2 cm^2) were prepared from "intact" skin (epidermis plus corium; thickness, 130 to 270 μm) and thin epidermal layers or corium layers (thickness, 60 to 90 μm). The specimens were attached with their inner (or outer) surfaces to the grounded electrode of a sample holder and were investigated in a shielded sample chamber. The front electrode (area, 10 mm^2) was identical to the measuring electrode used for the in vivo measurements.

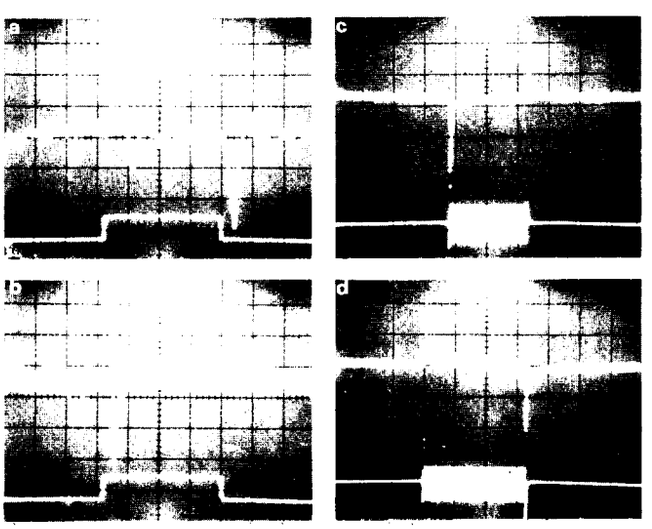

Fig. 1. Polar behavior of epidermis of human skin (fresh preparations). Opposite signs of PZE (a and b) and PE (c and d) voltage responses of outer (a and c) and inner (b and d) surfaces of the skin sample. (a and b) PZE responses to a square uniaxial pressure pulse; upper trace, skin response (100 μV per division); lower trace, signal of frequency generator (27.0 Hz). (c and d) PE responses caused by dielectric heating (radio-frequency pulse of 12.6 MHz and ~ 200-msec duration); upper trace, skin response; lower trace, radio-frequency signal.

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