bation period labeled amino acids are incorporated into hormone, neurophysin, and many other cellular proteins and polypeptides (*2*, *4*). A two- to fourfold stimulation of vasopressin and neurophysin labeling was observed when the adult HNC cultures were labeled in the presence of 45-day-old fetal HNC tissues. Apparently unaffected was the labeling of acid-soluble (*p*H 1.0) and -insoluble proteins and the free amino acid pool of the adult cultures, as well as vasopressin and neurophysin of the fetal HNC tissues.

The possibility that the fetal HNC was releasing into the medium some factor that stimulates vasopressin and neurophysin biosynthesis in the adult tissue was examined in a number of ways. Adult guinea pig HNC cultured in media that had been conditioned by prior incubation with fetal HNC tissues for 12 to 24 hours also showed an apparent specific two- to fourfold stimulation of labeling of vasopressin and neurophysin in adult cultures (that is, incorporation of ^{3}H-labeled amino acids into other measured protein fractions was not affected significantly). Furthermore, labeled hormone is not found in significant quantities in either control or stimulated labeling media. In these experiments, a clear concentration-response relation, however, could not be demonstrated since serial dilutions of conditioned media were equally effective until a critical dilution is reached beyond which the stimulatory effect is no longer observed. The fetal factor that influences vasopressin metabolism in adult HNC cultures can be demonstrated only during a limited period of gestation. Medium conditioned with the HNC explanted from fetuses 33 days old shows no stimulatory effect, nor does medium conditioned with tissue from 56-day-old fetuses (bars in Fig. 1). Thus, the factor appears to be specific for the period during which the neurosecretory neurons undergo their most rapid phase of vasopressin and neurophysin biosynthesis and storage. Further specificity of the factor is demonstrated by the fact that among media conditioned by either fetal HNC, liver, or cerebral cortex (all from fetuses of 45 days gestation), or adult HNC, only the first stimulates incorporation of ^{3}H-labeled amino acids into vasopressin (Fig. 2).

Further evidence for the presence of a "stimulatory factor" contained within the fetal HNC was obtained by preparing extracts of such fetal tissues. Freshly excised HNC tissues (42 to 47 days of gestation) were homogenized in ice-cold, phosphate-buffered saline and centrifuged at 100,000*g* for 2 hours. Addition of this soluble fetal HNC extract to the standard labeling medium again led to a two- to fourfold stimulation in the incorporation of radioactive amino acids into vasopressin of the adult HNC organ cultures. Again, this stimulation occurs in the absence of significant changes in the incorporation of labeled amino acids into total protein; similar extracts prepared from fetal liver and cortex had no effect. The complexity of the assay for the stimulatory factor (isolation of isotopically pure, labeled vasopressin or neurophysin, or both) has made its physical and biological characterization difficult. Our initial fractionation studies indicate that the factor is excluded from Sephadex G-25, but included in Sephadex G-75, suggesting a molecular weight in the 5,000 to 70,000 range, or alternatively that it is associated with other molecules in this range. Further preliminary studies suggest that the factor is sensitive to the action of trypsin and is thus polypeptide in nature.

The existence of a factor specifically in the fetal hypothalamus, which stimulates synthesis of vasopressin in adult HNC organ cultures, may provide a useful tool for further elucidation of mechanisms involved in the neurosecretory process. The fact that the factor is present only in the gestation period during which the vasopressin neurosecretory system is rapidly developing adds to its interest and may have important implications in the developmental process.

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References and Notes

1. H. Sachs, R. Goodman, J. Osinchak, J. McKelvy, *Proc. Natl. Acad. Sci. U.S.A.* **68**, 2782 (1971).
2. D. Pearson, A. Shainberg, J. Osinchak, H. Sachs, *Endocrinology*, in press.
3. D. Pearson, A. Shainberg, S. Malamed, H. Sachs, *ibid.*, in press.
4. A. Nureddin, S. Shin, H. Sachs, *ibid.*, in press.
5. S. Donev, *Z. Zellforsch.* **104**, 517 (1970).
6. R. Perks, *Nature (Lond.)* **223**, 1169 (1969).
7. W. H. Sawyer, in *The Pituitary Gland*, G. Harris and B. T. Donovan, Eds. (Univ. of California Press, Berkeley, 1966), vol. 3, p. 288.
8. We gratefully acknowledge the assistance of Dr. Wilbur Sawyer of Columbia University who carried out the vasotocin, antidiuretic, and oxytocic bioassays, as desribed in (*7*), p. 317.
9. S. Udenfriend, S. Stein, P. Bohlen, W. Dairman, W. Leimgruber, W. Weigele, *Science* **178**, 871 (1972).
10. We thank Mr. L. Brink and Ms. Diana Matuszewski for excellent technical assistance.
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Absence of Pyruvate Decarboxylase Activity in Man: A Cause of Congenital Lactic Acidosis

Abstract. *A complete deficiency in the pyruvate dehydrogenase system activity contributed to the death of a 6-month-old infant with congenital lactic acidosis. The enzymatic block could be isolated to the first component, pyruvate decarboxylase (E_1) of the pyruvate dehydrogenase complex. This enzymatic deficiency allowed a demonstration of an "intercomplex" exchange of the components of the mammalian pyruvate dehydrogenase system and indicated that the first component is normally present in an apparent excess.*

The clinical syndrome congenital lactic acidosis is not a single disease entity but rather may be caused by a variety of genetically determined enzyme deficiencies including pyruvate carboxylase (E.C. 6.4.1.1) (*1*, *2*), fructose-1,6-diphosphatase (hexose diphosphatase E.C. 3.1.3.11) (*3*), and pyruvate dehydrogenase (lipoate) (E.C. 1.2.4.1).

Pyruvate dehydrogenase (PDH) is a multienzyme system that can be purified and separated into at least three separate components and then reconstituted from the individual components (*4*, *5*). The three enzymes comprising the PDH complex are pyruvate decarboxylase (E_1) (E.C. 4.1.1.1), dihydrolipoyl transacetylase (E_2) (E.C. 2.3.1.12), and dihydrolipoyl dehydrogenase (E_3) (E.C. 1.6.4.3); they exist in a molecular ratio of 12 : 1 : 6 (*4*). Pyruvate decarboxylase in vivo exists in an active and inactive form (*6*, *7*). The conversion from the active to the inactive form is catalyzed by the kinase, pyruvate decarboxylase:ATP transphosphorylase, with the conversion from

the inactive to the active form being catalyzed by a magnesium dependent phosphatase, pyruvate decarboxylase phosphate phosphatase. The interconversion is reversible and is believed to be controlled by the metabolic state of the animal through cyclic adenosine monophosphate (*6, 7*). Because of its central role in carbohydrate metabolism, a deficiency in PDH activity in man, resulting from a genetic mutation, would cause varying degrees of metabolic abnormalities including elevated concentrations of pyruvate, lactate, and alanine in the serum, and varying degrees of associated physical abnormalities. A partial deficiency of pyruvate metabolism caused by a deficiency of E_1 activity ($\approx$ 15 to 20 percent of normal) has been reported in an 8-year-old child who had intermittent attacks of ataxia during times of febrile illnesses or other stress (*8, 9*). A more seriously affected patient was a 3-year-old child with diffuse neurologic involvement including microcephaly, blindness, motor disturbances, and mental retardation. Although this child had a greatly reduced total PDH system activity ($\approx$ 15 to 30 percent of normal), her E_1 activity was within normal limits, an indication of a deficiency in some other aspect of the complex, presumably either E_2 or E_3 activity (*10*).

Our study was made on an infant who was born after 35 weeks of gestation and weighed 1.32 kg at birth. He showed rapid respirations and several abnormal neurological signs during the first days of life. His blood *p*H was 7.32 with a partial pressure of CO_2 of 10 mm-Hg and bicarbonate of 6 meq/liter. The plasma pyruvate was 0.65 m*M* (the normal being 0.03 to 0.1 m*M*). He was considered to have congenital lactic acidosis and died at 6 months of age after a progressively downward clinical course.

The clinical course, which included serum lactic acid concentration correction by dietary manipulations, suggested impairment of carbohydrate metabolism at the level of the PDH complex. Liver and brain tissues were obtained at autopsy, stored frozen at −70°C, and later used in analysis of enzyme activity. In whole homogenates of these tissues, there was no measurable PDH complex activity or E_1 activity (Table 1). However, E_2 and E_3 activity in liver and brain homogenates were within normal limits.

A series of mixing experiments designed to eliminate the possibility of an endogenous inhibitor demonstrated an apparent interaction of the components of the PDH complex of normal and affected tissues during the 10-minute incubation by as much as a threefold increase in measured enzymatic activity compared with the expected activity (Table 2). Denaturation by heat or ethanol of either homogenate, from the tissues of the affected child or an appropriate control prevented the seeming activation of the PDH complex, suggesting that an interaction of labile protein constituents and not a stable "cofactor" was responsible for the "activation." In addition, after mixing there was no increase in the apparent activity of the individual components of the PDH complex as assayed separately (data not shown) (*5*). Preliminary incubation, either before or after mixing with 5 m*M* Mg^{2+}, failed to increase any of the individual enzymatic activities. This concentration of Mg^{2+} was sufficient for conversion of inactive phosphorylated pyruvate decarboxylase (E_1) to the active dephospho form in purified heart and kidney preparations and in brain homogenates (*6, 11*). Therefore, the apparent activation of the mutant PDH complex by normal material was not due to activation of any of the individual enzymes, lack of the activating enzyme (pyruvate decarboxylase phosphate phosphatase), lack of a soluble cofactor, or presence of an inhibitor; but most probably it was due to an interaction of the individual components of the mutant and normal PDH complexes to form mixed functioning complexes. These mixing experiments also suggest that in vivo the E_1 component in the PDH complex is not the limiting portion of the complex, but rather appears to be present in excess.

Gounaris and Hager (*12*) used mixing experiments to show that extracts from wild-type *Escherichia coli* could supply an enzyme component, E_1, of

Patient	Diagnosis	Activity (μmole/hour per mg protein)	
		PDH complex	E_1
	Liver		
1	5 months SID	0.139	2.04×10^{-3}
2	2 months SID	0.143	1.83×10^{-3}
3	3 months SID	0.126	1.31×10^{-3}
4	6 months propositus	< 0.005	$< 0.01 \times 10^{-3}$
	Brain		
1	6 years AE	0.08	3.29×10^{-3}
2	4 years MLD	0.11	6.03×10^{-3}
3	16 years BD	0.11	3.54×10^{-3}
4	6 months propositus	< 0.005	$< 0.01 \times 10^{-3}$

Table 1. Pyruvate dehydrogenase complex and pyruvate decarboxylase activity in liver and brain homogenates. The PDH complex was assayed by a modification of a previously described method (*11*). The incubation mixture (1 ml) contained 48 m*M* phosphate buffer, *p*H 7.3, 9 m*M* dithiothreitol, 6 m*M* NAD^+, 2 m*M* thiamine pyrophosphate, 0.1 m*M* coenzyme A, 2 m*M* [1-^{14}C]pyruvate, 125 μg of lactate dehydrogenase, 5 μg of phosphotransacetylase, and liver or brain homogenates in amounts that yielded linear reaction rates over the assay period. Incubation was carried out at 37°C for 10 minutes, and the reaction was stopped by the addition of 12 percent trichloroacetic acid; during the 10-minute incubation period approximately 5 to 10 percent of the substrate was hydrolyzed. Omission of coenzyme A reduced the PDH activity of the controls to near zero. The pyruvate decarboxylase (E_1) activity was assayed by the method of Reed and Willms (*5*), modified to use sodium [1-^{14}C]pyruvate. The reaction mixture was incubated at 37°C for 3 hours. In both enzyme assays the evolved $^{14}CO_2$ was collected in hyamine hydroxide at 37°C for 45 minutes, and the radioactivity was determined by liquid scintillation spectrophotometry; SID, sudden infant death syndrome; AE, anoxic encephalopathy; MLD, metachromatic leukodystrophy; BD, Batten's disease.

Table 2. Pyruvate dehydrogenase complex and pyruvate decarboxylase activity in mixtures of liver homogenates of affected and control subjects. The results represent several individual determinations. The methods are the same as those used in Table 1; E, experimental; C, calculated.

Protein added (mg)		Pyruvate dehydrogenase complex (μmole/hour per mg protein)		Ratio E/C	Pyruvate decarboxylase (E_1) (μmole/hour per mg protein)		Ratio E/C
Control	Propositus	E	C*		E	C*	
1.20	0	0.139			2.04×10^{-3}		
1.08	0.12	0.151	0.125	1.20	1.89×10^{-3}	1.83×10^{-3}	1.03
0.60	0.60	0.133	0.069	1.93	1.08×10^{-3}	1.02×10^{-3}	1.06
0.30	0.90	0.088	0.039	2.25	0.44×10^{-3}	0.51×10^{-3}	0.88
0.12	1.08	0.047	0.014	3.40	0.19×10^{-3}	0.20×10^{-3}	0.93
0	1.20	< 0.005			$< 0.01 \times 10^{-3}$		

* The calculated specific activity assumes the simple addition or the arithmetical sum of the specific activity of the control sample and the patient sample prior to mixing.

the PDH system to reconstitute the activity of a strain of *E. coli* deficient in PDH and that E_1 was present in excess in the wild type. The possibility of a similar such "intercomplex" exchange but involving E_3 has been proposed for a mammalian PDH system (*13*). Our experiments indicate that in a mammalian PDH system, "intercomplex" exchanges probably can occur and that pyruvate decarboxylase (E_1) in normal tissues appears to be present in excess. It is not certain whether the mobile, exchangeable, component is E_1, E_3, or all three. Normal levels of E_2 and E_3 with an absence of E_1 in both the *E. coli* mutant (*13*) and in the case we studied suggest that the levels of E_1, its substrates, or products do not have a modifying influence upon the level of the other two enzymes.

The total absence of PDH activity in the 6-month-old child resulted in severe physical and neurologic deficits leading to death at 6 months of age. The ability of this infant to survive fetal life is remarkable and implies an adequate supply of noncarbohydrate metabolites for tricarboxylic acid cycle oxidation.

This apparent genetic mutation in the E_1 component of the PDH complex provides some insight as to the possible reason for the clinically variable nature of the two previous similar cases. In the 8-year-old child (*8*), the E_1 activity was approximately 15 to 20 percent of normal. From our experiments this activity would allow marginal acetyl coenzyme A formation from pyruvate, but would not allow increased production during periods of physiological stress. On the other hand, the 3-year-old child (*10*) had a residual total PDH complex enzymatic activity of approximately 15 percent of normal due to a deficiency of either E_2 or E_3, the apparent limiting components of the PDH system. Such a defect would result in a much greater total deficiency of carbohydrate derived acetyl coenzyme A, and this greater absolute deficiency would result in a more severely affected patient.

Enzymatic studies of similar genetic mutations in humans should contribute to a better understanding of this type of carbohydrate metabolism and its associated diseases.

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References and Notes

1. T. Yoshida, K. Tada, T. Konno, T. Arakawa, *Tohoku J. Exp. Med.* **99**, 121 (1971); M. G. Brunette, E. Delvin, B. Hazel, C. R. Scriver, *Pediatrics* **50**, 702 (1972).
2. The enzymes are called by their trivial names known to workers in this field. The systematic and other names can be obtained by referring to the E.C. numbers in parentheses.
3. K. Baerlocher, R. Gitzelmann, R. Nüssli, G. Dumermuth, *Helv. Paediatr. Acta* **26**, 489 (1971); A. S. Pagliara, I. E. Karl, J. P. Keating, B. I. Brown, D. M. Kipnis, *J. Clin. Invest.* **51**, 2115 (1972).
4. T. C. Linn, J. W. Pelley, F. H. Pettit, F. Hucho, D. D. Randall, L. J. Reed, *Arch. Biochem. Biophys.* **148**, 327 (1972); M. Koike, L. J. Reed, W. R. Carroll, *J. Biol. Chem.* **238**, 30 (1963).
5. L. J. Reed and C. R. Willms, *Meth. Enzymol.* **9**, 247 (1966).
6. O. Wieland and E. Siess, *Proc. Natl. Acad. Sci. U.S.A.* **65**, 947 (197(———, F. H. Schultze-Wethmar, H. G. v. Funcke, B. Winton, *Arch. Biochem. Biophys.* **143**, 593 (1971).
7. E. J. Walajtys, D. P. Gottesman, J. R. Williamscn, *J. Biol. Chem.* **249**, 1857 (1974).
8. J. P. Blass, J. Avigan, B. W. Uhlendorf, *J. Clin. Invest.* **49**, 423 (1970).
9. J. P. Blass, A. P. Kark, W. K. Engle, *Arch. Neurol.* **25**, 449 (1971).
10. J. P. Blass, J. D. Schulman, D. S. Young, E. Horn, *J. Clin. Invest.* **51**, 1845 (1972).
11. J. E. Cremer, H. M. Teal, *FEBS (Fed. Eur. Biochem. Soc.) Lett.* **39**, 17 (1974).
12. A. D. Gounaris and L. P. Hager, *J. Biol. Chem.* **236**, 1013 (1961).
13. C. R. Barrera, G. Namihira, L. Hamilton, P. Munck, M. H. Eley, T. C. Linn, L. J. Reed. *Arch. Biochem. Biophys.* **148**, 343 (1972).
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Pyridoxal Kinase: Decreased Activity in Red Blood Cells of Afro-Americans

Abstract. *The mean pyridoxal kinase activity in red blood cells of American blacks was approximately 50 percent lower than that of American whites. Lymphocytes, granulocytes, and cultured skin fibroblasts from black and white donors contained identical pyridoxal kinase activity. The pyridoxal kinase of blacks was indistinguishable from that of whites with respect to heat stability, chromatographic mobility on microgranular diethylaminoethyl cellulose, Michaelis-Menten constant for pyridoxine, and susceptibility to inhibition by 4-deoxypyridoxine. The difference of the activity of this enzyme in whites and in blacks is much greater than any previously observed biochemical difference between the races.*

There are many anthropometric differences and differences in skin pigment which define the races of man. However, no such diversity is apparent in biochemical measurements. There are, of course, many polymorphisms that are present exclusively, or are largely confined to, a single race. However, differences in the activity of an enzyme affecting most members of a race are quite unusual. While the average galactokinase activity (*1*) and average adenosine triphosphate concentration (*2; 3*, pp. 92–94) of the red blood cells of blacks have been shown to be significantly lower than that of whites, the differences observed have been relatively small, with considerable interracial overlap. We now report a striking difference in the activity of red cell pyridoxal kinase (E.C. 2.7.1.35) of whites and blacks. This enzyme catalyzes the phosphorylation of pyridoxine and pyridoxal, an essential step in the conversion of pyridoxine to its active coenzyme form. The difference in this activity seems to be by far the greatest interracial difference in the activity of

Table 1. Pyridoxal kinase activity in blood cells and skin fibroblasts from American black and white subjects. Pyridoxal kinase activity in red cells is expressed as milliunits per gram of Hb; that of lymphocytes, granulocytes, and fibroblasts as milliunits per gram of protein. Values are given as mean ± standard deviation, with ranges of values shown on the line below. Single numbers in parentheses are the number of samples examined.

Cells	Race	
	Black	White
Red cells	0.676 ± 0.282 (25) (0.310–1.569)	1.337 ± 0.286 (36) (0.669–2.024)
Lymphocytes	30.50 ± 12.47 (6) (10.99–47.20)	30.12 ± 9.41 (6) (17.55–41.05)
Granulocytes	47.28 ± 6.91 (6) (36.45–56.55)	49.52 ± 13.85 (6) (35.42–69.66)
Fibroblasts	60.40 (2) (33.16, 87.63)	62.27 (2) (46.58, 77.95)