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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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)

any enzyme that has been observed up to the present time. With the exception of the Duffy blood group marker and to a lesser extent certain other red cell antigens (*4*), no other biochemical differences between the races approaches the magnitude of the difference observed.

Freshly drawn venous blood from healthy adults living in the Los Angeles area was collected in acid-citrate-dextrose (ACD) solution (*3*, p. 10) and was filtered on a sulfoethyl cellulose–Sephadex G-25 column to remove white blood cells and platelets (*5*). The red cells were washed twice with 0.154*M* NaCl solution. One volume of washed red cells was diluted with nine volumes of water to prepare hemolyzates. Samples from white and black donors or subjects from other races were always assayed concurrently. Pyridoxal kinase activity in red cells was determined with [^{3}H]pyridoxine as the substrate, and the unreacted pyridoxine was adsorbed on Dowex-50 (*6*). The specific activity of the enzyme was expressed as nanomoles of pyridoxine phosphate formed per minute per gram of hemoglobin (mU per gram of Hb).

Red cells from 36 American white donors contained a mean pyridoxal kinase activity of 1.337 ± 0.286 mU per gram of Hb while red cells from 25 American blacks contained 0.676 ± 0.282 mU per gram of Hb. The distribution of pyridoxal kinase activities in red blood cells from persons of these two groups and other races is shown in Fig. 1. Nine Filipinos and seven Asian Indians had mean pyridoxal kinase activites of 1.074 ± 0.217 and 1.119 ± 0.169 mU per gram of Hb, respectively. The 12 Orientals, Chinese and Japanese, possessed a mean pyridoxal kinase activity of 1.221 ± 0.339 mU per gram of Hb in their red cells. American blacks had the lowest red cell pyridoxal kinase activity among different races examined. Within the group classified as "black," there was no obvious correlation between skin color and red cell enzyme activity.

Pyridoxal kinase assays were carried out on lymphocytes and granulocytes from the same individuals, the cells being separated by centrifugation on Ficoll-Hypaque (*7*) which was followed, in the case of granulocytes, by shock osmotic lysis. Pyridoxal kinase activities in skin fibroblasts obtained from white and black persons were also determined (see Table 1). These activities indicate that the deficiency of pyridoxal kinase activity in blood samples of blacks is limited to the red cells. No correlation between enzyme activities in red cells with those in lymphocytes and granulocytes was found.

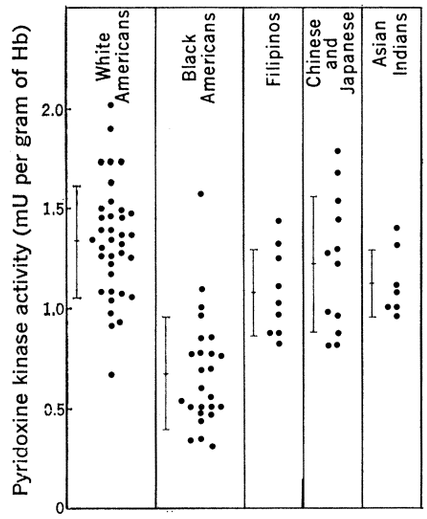

Fig. 1. The distribution of individual pyridoxal kinase in red blood cells from different races. The vertical bars indicate the population means and standard deviations.

The possibility that factors such as red cell age or pyridoxal phosphate (PLP) concentrations might influence the pyridoxal kinase activity in these cells was examined by studying erythrocyte glutamic–oxaloacetic transaminase (EGOT) and hexokinase activities as indicators of red cell age (*3*, pp. 38–40 and pp. 75 and 76), and activation of EGOT by PLP in red cells as an indicator of PLP concentrations (*8*). The mean hexokinase activity in red cells of 25 blacks was 0.514 ± 0.079 and that of 23 whites 0.477 ± 0.110 unit per gram of Hb. The red cells of 25 black donors contained a mean EGOT activity of 3.13 ± 0.633 units per gram of Hb with 57.82 ± 16.75 percent activation of EGOT activity when it was stimulated with 0.02 m*M* PLP; red cells from 23 white donors had a mean EGOT activity of 3.17 ± 0.557 units per gram of Hb with 58.63 ± 18.56 percent stimulation of EGOT by PLP. These results indicate that there is no obvious difference between red cell age or PLP content of black and white persons.

In an effort to clarify the molecular basis of the low pyridoxal kinase activity in the red cells of black subjects, some biochemical properties of the red cell enzymes from blacks and whites were investigated. The heat stability of hemolyzate pyridoxal kinase at *p*H 6.5 and 45°C and the chromatographic mobility on a microgranular diethylaminoethyl cellulose (DE-52; Whatman) column (*9*) of pyridoxal kinase from black and white subjects were found to be alike. No significant difference of Michaelis-Menten constant (K_m) for pyridoxine between these two races was observed. The mean K_m for hemoglobin-free pyridoxal kinase prepared by DE-52 chromatography (*10*) from five whites was 2.79 ± 0.69 m*M* and that from five blacks with pyridoxal kinase activities lower than 0.509 mU per gram of Hb was 2.07 ± 0.75 m*M*. The effect of 4-deoxypyridoxine, a potent inhibitor of pyridoxal kinase, on the enzyme activity was tested at concentrations from 8.3 to 417 μM at *p*H 7.0. A similar degree of inhibition on hemolyzate and hemoglobin-free pyridoxal kinases from blacks and whites was observed. Assays carried out on mixed hemolyzates from white and black donors gave the calculated activity without any evidence of inhibition or activation. Dialysis of hemolyzates did not affect their activity.

It seems almost certain that the racial difference in pyridoxal kinase activity which we have observed exists on a genetic basis. While the activity of at least one red cell enzyme, glutathione reductase, is markedly influenced by diet (*11*), most of the blood samples obtained in this study were from hospital employees including black physicians, nurses, and administrators, whose diet does not differ materially from that of their white colleagues. In addition, it has been demonstrated that vitamin B_6 deficiency has little effect on pyridoxal kinase activity in rat tissues (*12*).

Although we have not demonstrated a qualitative abnormality of red cell pyridoxal kinase between black and white subjects, the most simple genetic explanation of our data is that a structural gene mutation coding for an enzyme of approximately one-third of the usual activity has reached a population frequency approaching 1.0 in African populations. The Afro-American population studied consists largely of persons homozygous for the original low-activity gene or those heterozygous for this gene. Alternatively, the possibility of multigenic inheritance deserves consideration.

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29 October 1974

Inhibition of Catecholamine Release by Tolbutamide and Other Sulfonylureas

Abstract. *Tolbutamide and other sulfonylureas inhibited spontaneous and nicotine-induced release of catecholamines from the perfused cat adrenal gland and nicotine-induced release of [³H]norepinephrine from isolated guinea pig hearts. Of the sulfonylureas tested, the order of potency of this inhibitory effect paralleled the hypoglycemic action. These results raise the possibility that the inhibition of the sympathoadrenal system may contribute in part to the hypoglycemic action of sulfonylureas.*

Sulfonylureas are oral hypoglycemic agents which have been utilized extensively in the treatment of adult-onset diabetes mellitus. The primary action of this group of agents has been attributed to the direct stimulation of pancreatic beta cells to release insulin (*1*). However, the poor correlation between their insulin-releasing effect and diabetic control has led to speculation of extrapancreatic actions of sulfonylureas (*2*). Herein we report an effect of tolbutamide and other sulfonylureas on the spontaneous and nicotine-induced release of catecholamines from isolated cat adrenal glands (*3*) and on nicotine-induced release of [³H]norepinephrine ([³H]NE) from isolated guinea pig hearts (*4*). These observations suggest that sulfonylureas have a direct inhibitory action on the sympathoadrenal system.

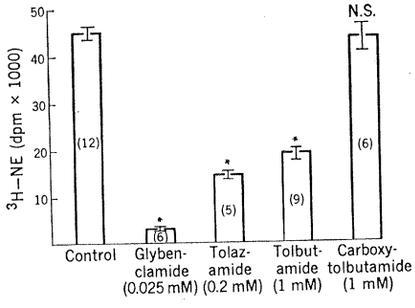

Fig. 1. The effects of four sulfonylureas on the release of [³H]NE induced by nicotine in isolated guinea pig hearts, measured in disintegrations per minute. Release of [³H] was stimulated by a single injection of nicotine. Five minutes before and after injection, one of the sulfonylureas or the vehicle used to dissolve sulfonylureas (control) was present. Asterisk denotes difference from control is significant at $P < .001$. *N.S.*, not significant.

Isolated cat adrenal glands were retrogradely perfused with phosphate-buffered Locke's solution, and the adrenal catecholamine secretion was monitored by a modification of the procedure of Robinson and Watts (*5*). This experimental procedure has been reported in detail previously (*6*). The effect of tolbutamide on the spontaneous secretion of catecholamines was observed by perfusing the glands with Locke's solution containing tolbutamide (0.1, 0.3, or 1 m*M*) for 5 minutes. To study the effect of sulfonylureas on the nicotine-induced release of catecholamines, each gland was stimulated twice by perfusion with nicotine ($10^{-6}M$) for 1 minute. Five minutes before and 5 minutes after the first stimulation by nicotine, the adrenal was perfused with either tolbutamide (0.3 or 1 m*M*) or tolazamide (0.2 m*M*). The administration of nicotine was repeated 30 minutes after termination of the perfusion with sulfonylureas and served as a control response.

Endogenous norepinephrine stores in isolated guinea pig hearts were prelabeled with [³H]NE. Hearts were stimuated to release [³H]NE by single injections of nicotine (4×10^{-7} mole) 20 minutes after [³H]NE labeling. The perfusate effluents from the hearts were continuously collected and analyzed for [³H]NE by liquid scintillation spectrometry (*7*). To study the effect of sulfonylureas on nicotine-induced release of myocardial [³H]NE, sulfonylureas (tolbutamide, 1 m*M*; carboxytolbutamide, 1 m*M*; tolazamide, 0.2 m*M*; glybenclamide, 0.025 m*M*) were individually added to the perfusion fluid from 5 minutes before to 5 minutes after the injection of nicotine.

The spontaneous output of catecholamines from the cat adrenal gland usually became stable 1 hour after the initiation of perfusion with Locke's solution. Tolbutamide (0.3 or 1 m*M*) caused a decline in spontaneous catecholamine output (see Table 1). This inhibitory effect of tolbutamide was reversible. Nicotine-induced release of adrenal catecholamines was also suppressed by tolbutamide. When adrenal glands were stimulated consecutively at 30-minute intervals by the same dose of nicotine ($10^{-6}M$ for 1 minute), the response to the second stimulation was only 60 to 70 percent of the initial response (data not shown). When tolbutamide (0.3 or 1 m*M*) was present only during the initial exposure to nicotine, the second response to nicotine was then greater than the first. Tolazamide (0.2 m*M*) exerted a similar inhibitory action (see Table 1).

Nicotine-induced release of [³H]NE from the isolated guinea pig hearts was also inhibited by several sulfonylureas (tolbutamide, tolazamide, and glybenclamide). The order of potency in inhibiting catecholamine release by these

Table 1. Effect of tolbutamide and tolazamide on spontaneous and nicotine-induced release of total catecholamines from isolated cat adrenal glands. Each gland served as its own control. The data are expressed as percent of control. The number of glands studied is indicated in parentheses after the mean ± S.E.M.

Drugs (m*M*)	Catecholamines released (% of control)	
	Spontaneous*	Nicotine-induced†
Tolbutamide		
0.1	95 ± 2 (3)	
0.3	72 ± 6 (5)‡	77 ± 5 (4)‡
1.0	62 ± 8 (5)‡	56 ± 10 (7)‡
Tolazamide		
0.2		51 ± 7 (3)‡

* [Rate of spontaneous output in the presence of sulfonylurea (ng/min) divided by the rate of spontaneous output in the absence of sulfonylurea (ng/min)] × 100. Control spontaneous output was 230 ± 30 ng/min. † [Nicotine-induced release in the presence of sulfonylurea (ng) divided by nicotine-induced release in the absence of sulfonylurea (ng) × 100. Control nicotine-induced release was 12,210 ± 1,330 ng. ‡ Signifiant at $P < .01$.