Evidence for Four Types of Erythrocyte Glucose-6-Phosphate Dehydrogenase from G-6-PD-deficient Human Subjects *

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Hereditary deficiency of glucose-6-phosphate dehydrogenase (G-6-PD) in the erythrocyte is generally characterized by a marked increase in hemolysis after administration of various compounds such as primaquine, sulfonamides, and other drugs (1–3). In some instances, a chronic hemolytic condition is observed in the absence of drug administration (4, 5). The former state is classed as drug sensitivity and the latter as congenital nonspherocytic hemolytic anemia (CNSHA) (3–5). The observed low level of G-6-PD catalytic activity may be attributed to either a quantitative difference in enzyme concentration or to a qualitative abnormality of the enzyme in the deficient patient (3, 6).

Characterization of the erythrocyte G-6-PD from normal and drug-sensitive male Negro patients has been reported (7–17). The enzyme from the drug-sensitive Negro male was indistinguishable from that of normal Negro males with respect to substrate and inhibitor affinity, pH optimum, and general stability (7).

Nonanemic Caucasian G-6-PD-deficient subjects exhibit qualitative enzyme abnormalities characterized by abnormal Michaelis constants (K_m) for glucose-6-phosphate (G-6-P) and 2-deoxyglucose-6-phosphate (2-d–G-6-P) (9, 11, 18). Abnormal electrophoretic mobility and thermal stability have also been observed in some subjects. Similarly, CNSHA subjects have demonstrated abnormalities ranging from only decreased thermostability to high G-6-P K_m values and varied electrophoretic mobilities suggesting the existence

of a number of different qualitative enzyme abnormalities (18, 19).

Male Negroes have either an electrophoretically fast moving G-6-PD (A) or a slower moving G-6-PD (B). Since normal Caucasians possess only the slower B G-6-PD, this is generally referred to as the normal enzyme. Drug-sensitive male Negro subjects have either a fast G-6-PD mobility that cannot be distinguished by electrophoresis from A or, rarely, a slow G-6-PD mobility that cannot be distinguished from normal G-6-PD (B). Caucasian deficient and CNSHA subjects may have either a fast mobility comparable to A, a normal slow mobility comparable to B, or a slower mobility than B (18).

Of particular interest is the variation in the K_m reported for the normal enzyme. Although 2.1×10^{-6} mole per L and 3.9×10^{-5} mole per L are the K_m values commonly reported for nicotinamide adenine dinucleotide phosphate (NADP) and G-6-P, respectively (12–14), some investigators have reported higher values (7, 11, 14, 17). The reported activation of drug-sensitive erythrocyte G-6-PD by normal stroma (8) has not been fully substantiated (7).

In the present investigation, erythrocyte G-6-PD from normal and deficient male subjects has been characterized in detail. The results suggest the classification of the deficient Negro into at least three groups with respect to G-6-PD characteristics. One group is indistinguishable from the normal enzyme (quantitative difference), whereas the other two show marked variation (qualitative difference). The G-6-PD from the subjects with CNSHA differs qualitatively from normal G-6-PD.

Methods

Assay procedure. The assay for G-6-PD was performed by following the change in absorbancy at 340 mµ

at convenient intervals (5 seconds) in a Beckman DU spectrophotometer adapted for recording and scale expansion (Gilford). Temperature control was obtained by circulating water from a thermostat through thermospacers at both ends of the cell compartment. Unless otherwise stated, NADP reduction was measured at 25° C as a function of time in a system consisting of 300 μmoles Tris buffer, pH 8.0, 20 μmoles MgCl$_2$, 1.8 μmoles G-6-P, 0.6 μmole NADP, and enzyme and water to a total volume of 3 ml. The light path was 1.0 cm. One unit of activity is defined as the amount of enzyme generating 1.0 μmole of NADPH (absorbancy 6.2) per minute under conditions of the assay. Specific activity is defined as the units of enzyme activity per milligram of protein. Protein was determined by the spectrophotometric method of Warburg and Christian (20).

Enzyme purification. The enzyme from the erythrocytes of each individual was purified according to the procedure of Kirkman (13) incorporating the DEAE-cellulose adsorption method of Chung and Langdon (12). The G-6-PD from nondeficient subjects could be purified to a specific activity of 60 to 70, whereas that
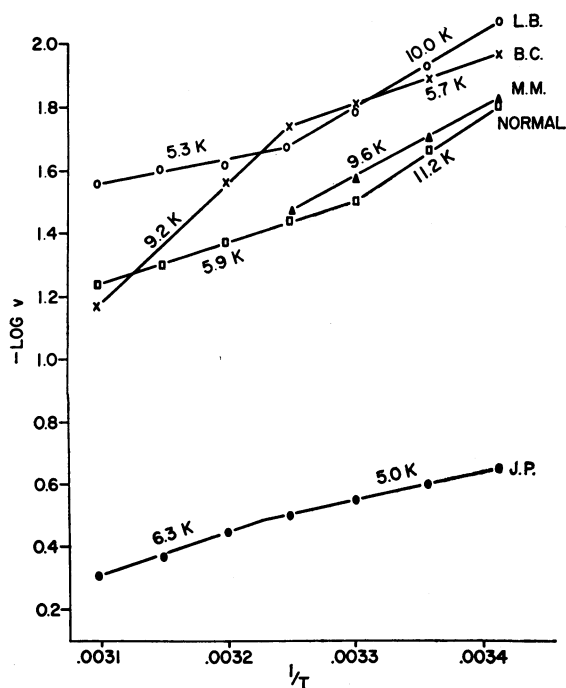

FIG. 1. VARIATION OF LOG VELOCITY WITH THE RECIPROCAL OF ABSOLUTE TEMPERATURE FOR THE NORMAL ENZYME AND DEFICIENT ENZYMES. The system consisted of 300 μmoles Tris buffer, pH 8.0, 20 μmoles MgCl$_2$, 0.6 μmole NADP, 1.80 μmoles glucose-6-phosphate (G-6-P), and enzyme and water to a total volume of 3.0 ml. The activation energy (E$_a$) for M.M. above 35° C could not be determined due to thermal lability of the enzyme. Each point is the average of two determinations. K = kilocalories per mole.

from deficient subjects could be purified to only 0.5 to 1.0. Ammonium sulfate was removed by dialysis before characterization of the purified enzyme. Since the normal enzyme from nondeficient subjects gave essentially the same data in each instance, the values have been pooled and presented in this paper as a range for the normal enzyme.

Materials. Substrates were obtained commercially.[1] The insoluble barium salts were converted to the sodium salts by treatment with AG 50W-X8 cation exchange resin.[2] The solution obtained was adjusted to pH 8.0 with NaOH before being made up to the 6 mM final concentration. The buffer solutions, Tris, glycine, and sodium phosphate, were adjusted to the desired pH with NaOH or HCl; with potassium phosphate buffer solution, KOH was employed. All other reagents were of analytical grade.

Electrophoresis. Vertical starch gel electrophoresis of red cell hemolysates was performed according to the method of Kirkman and Hendrickson (21). After electrophoresis, the starch gel was sliced horizontally and the dehydrogenase activity located according to the method of Chung and Langdon (12) using MTT [3] instead of Nitro-BT as the indicator dye.

Screening program. Fresh venous blood samples (20 ml) were collected in acid citrate dextrose (ACD) from 48 normal Caucasian males, 2 Caucasian males with CNSHA, and 42 normal and 8 G-6-PD-deficient Negro males. The samples were processed within 1 hour and duplicate spectrophotometric assays done immediately after centrifugation of the lysed cells. These results were qualitatively confirmed by the rapid methemoglobin reduction test (22) with complete correlation between both assay methods.

Fresh blood samples were centrifuged at 3,000 g for 10 minutes and the supernatant plasma and top layer containing leukocytes discarded. The packed erythrocytes were washed three times with 5 vol of physiological saline. A hematocrit reading was taken of the final saline suspension. The washed cells were then hemolyzed by mixing with 5 vol of a 7 mM solution of β-mercaptoethanol in distilled water. After 30 minutes the stroma was removed by centrifugation at 30,000 g for 20 minutes. The supernatant was assayed immediately for G-6-PD as described and the activity observed related directly to the red blood cell content as determined by the hematocrit.

Results

Erythrocyte G-6-PD levels. The G-6-PD activity in human erythrocytes from male Caucasian and Negro subjects is summarized in Table I. The normal range of G-6-PD activity is 100 to

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TABLE I

*G-6-PD activity in human erythrocytes from male Caucasian and Negro subjects**

Subject	Type	No. of subjects	Average	Range
			U activity/100 ml packed RBC	
Caucasian	Normal	48	115	100–135
Caucasian	CNSHA	H.E.	4	
Caucasian	CNSHA	M.M.	20	
Negro	Normal	42	117	100–160
Negro	Deficient	L.B.	41	
Negro	Deficient	F.H.	41	
Negro	Deficient	H.R.	41	
Negro	Deficient	E.E.	27	
Negro	Deficient	B.C.	24	
Negro	Deficient	N.M.	29	
Negro	Deficient	J.P.	41	
Negro	Deficient	L.M.†	41	

* G-6-PD = glucose-6-phosphate dehydrogenase; RBC = red blood cells; CNSHA = congenital nonspherocytic hemolytic anemia.

† Sample obtained was too small for further purification.

160 U per 100 ml packed red blood cells for both Negro and Caucasian subjects. The drug-sensitive Negro subjects had activity levels of 24 to 41 U per 100 ml packed red blood cells, whereas the CNSHA subjects had a range of 4 to 20.

TABLE II

Activation energy (E_a)* obtained for purified normal and deficient G-6-PD

Subject	E_a (20°–30° C)	E_a (40°–50° C)
	kcal/mole	*kcal/mole*
Normals (6)	9.7(9.0–11.4)	5.7(5.0–6.5)
Deficient Negroes		
L.B.	10.0	5.3
F.H.	5.4	10.4
H.R.	5.5	10.9
E.E.	5.0	9.8
B.C.	5.7	9.2
N.M.	6.0	11.0
J.P.	5.0	6.3
Deficient CNSHA		
H.E.	9.1	†
M.M.	9.6	†

* Each value is the average of two determinations. The variation with each sample was less than 5% of the mean.

† E_a at this temperature could not be determined due to the extreme thermal lability of the enzyme.

Activation energy. The effect of temperature on the velocity of the purified enzymes is illustrated in Figure 1. The average activation energy value, E_a, for the normal subjects and individual values for the deficient subjects are given in Table II. The average E_a values obtained for nor-
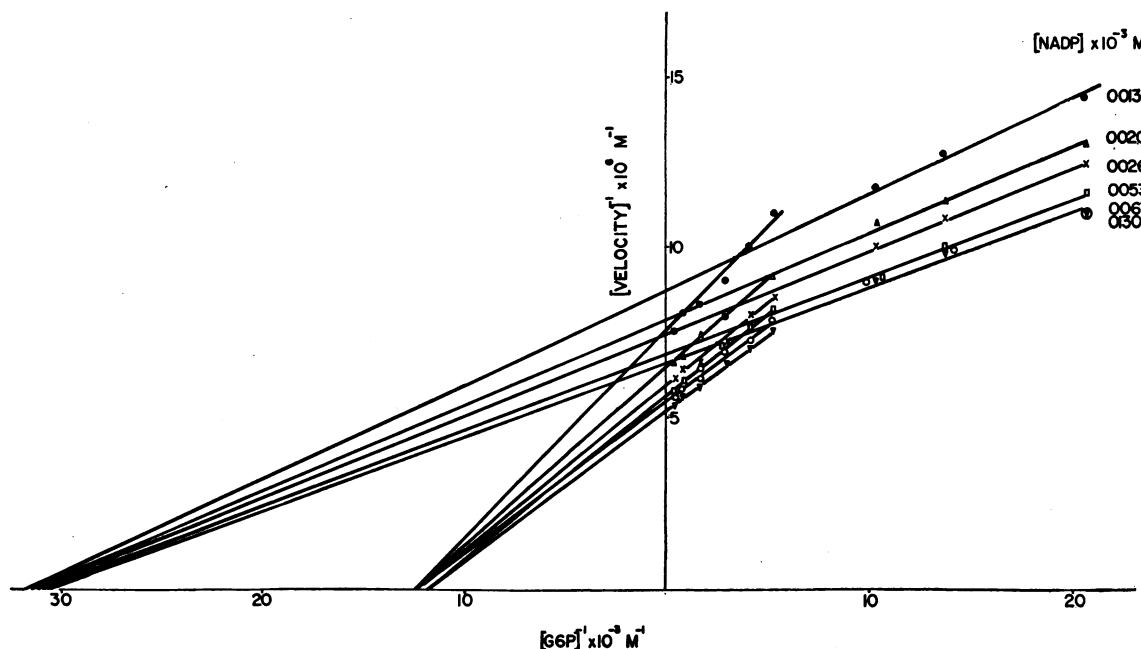

FIG. 2. LINEWEAVER-BURKE PLOTS OF THE FORWARD REACTION OF PURIFIED ERYTHROCYTE GLUCOSE-6-PHOSPHATE DEHYDROGENASE (G-6-PD) FROM A SINGLE NORMAL SUBJECT. The Figure illustrates the determination of the Michaelis constants (K_m) for G-6-P at five different concentrations of NADP employing purified G-6-PD from a single normal subject. The reaction mixture consisted of 300 μmoles of Tris buffer, pH 8.0, 20 μmoles MgCl₂, NADP and G-6-P as indicated, and enzyme and water to a total volume of 3.0 ml. Temperature, 25° C. Identical plots were obtained with deficient type I (L.B.). Deficient types II (F.H., H.R., E.E., B.C., N.M.) and III (J.P.) gave only a linear response over the same G-6-P concentration with a single K_m value corresponding to the lower value obtained with the normal enzyme.
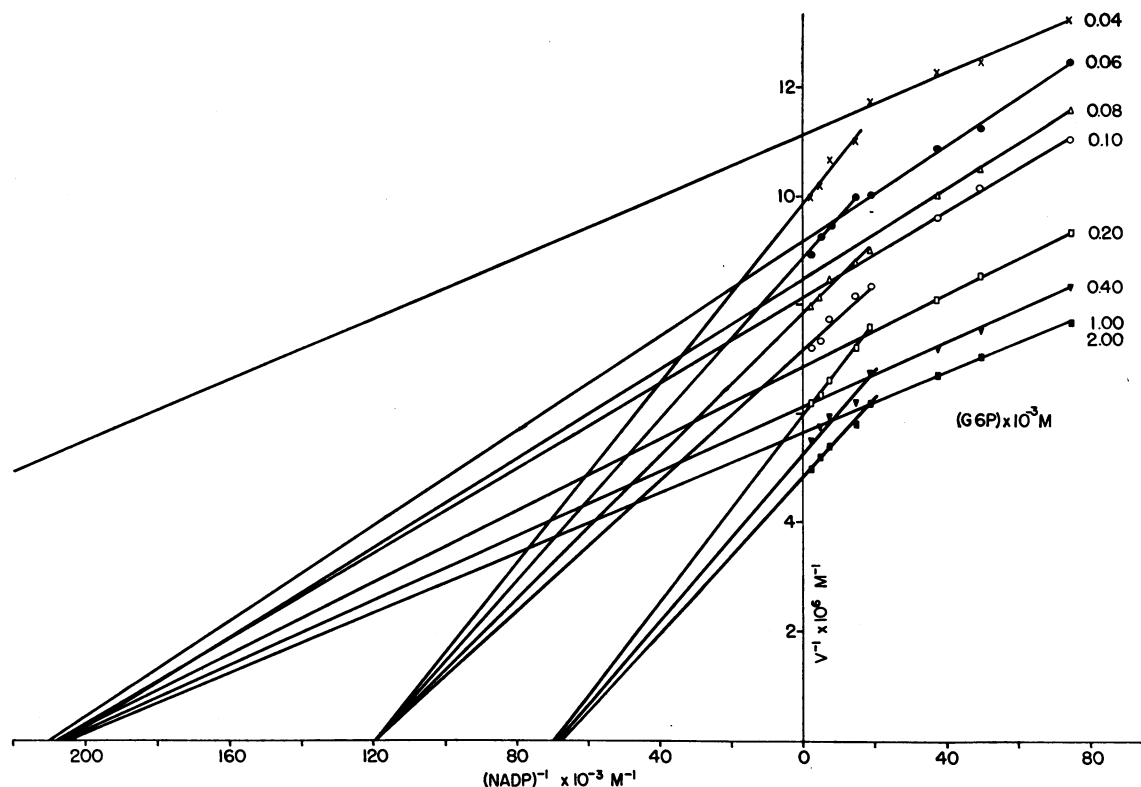
FIG. 3. LINEWEAVER-BURKE PLOTS OF THE FORWARD REACTION OF PURIFIED ERYTHROCYTE G-6-PD FROM A SINGLE NORMAL SUBJECT. The Figure illustrates the determination of the K_m for NADP at eight different concentrations of G-6-P employing purified G-6-PD from a single normal subject. The reaction mixture consisted of 300 μmoles of Tris buffer, pH 8.0, 20 μmoles $MgCl_2$, NADP and G-6-P as indicated, and enzyme and water to a total volume of 3.0 ml. Temperature, 25° C. Identical plots were obtained with deficient type I (L.B.). Deficient types II (F.H., H.R., E.E., B.C., N.M.) and III (J.P.) gave only linear responses over the NADP concentrations employed with K_m values corresponding to the two lower values obtained with the normal enzyme.

mal subjects were 9.7 kcal per mole below 30° C and 5.7 kcal per mole above 40° C. Subject L.B., like the normal subjects, gave an E_a of 10.0 kcal per mole below 30° C and 5.3 kcal per mole above 40° C. Subject J.P., however, had an E_a of 5.0 kcal per mole below 30° C, which is significantly lower than that of normal subjects, and 6.3 kcal per mole above 40° C. The remaining deficient Negro subjects, F.H., H.R., E.E., B.C., and N.M., had an E_a of approximately 5.0 kcal per mole below 30° C and 10.0 kcal per mole above 40° C, which is the reverse of the pattern obtained with normal subjects. An E_a of 9.3 kcal per mole below 30° C was found for CNSHA subjects, which is comparable to the normal subjects, but the E_a above 40° C could not be determined due to the extreme thermal lability of the enzyme.

Michaelis constants. The K_m values for G-6-P and NADP with G-6-PD were determined for

each of the subjects. Two K_m values for G-6-P (Figure 2) and four for NADP (Figure 3) are found for normal human erythrocyte G-6-PD. The values obtained for each deficient subject with substrate concentrations of 4×10^{-5} to 2×10^{-3} mole per L for G-6-P and 1.3×10^{-6} to 6.7×10^{-4} mole per L for NADP are given in Table III. Deficient subject L.B. is identical with the normal, but the remaining six deficient subjects demonstrate only the lower K_m values (one for G-6-P and two for NADP). The CNSHA subjects possess single G-6-P and NADP K_m values approximating the highest values obtained with the normals.

The degree of purity of the normal enzyme had no effect on the values of the kinetic parameters. Identical results were obtained for the normal enzyme whether enzyme of specific activity 0.5 or 70 was employed as well as with deficient subject

TABLE III

Michaelis constants for NADP and G-6-P for purified normal and deficient erythrocyte G-6-PD

	Range of substrate concentrations employed		Normals Range	Deficient Negroes							CNSHA	
Substrate	NADP	G-6-P		L.B.	F.H.	H.R.	E.E.	B.C.	N.M.	J.P.	H.E.	M.M.
	$M \times 10^{-3}$			$K_m \times 10^6$								
G-6-P	0.013–0.13	0.04–0.16	30–50	46	50	40	38	47	42	40	*	*
G-6-P	0.013–0.13	0.16–2.00	70–88	78	†	†	†	†	†	†	90	90
NADP	0.02–0.05	0.04‡	1.7–2.3	2.1	2.0	1.9	2.3	2.0	1.8	1.9	*	*
NADP	0.02–0.05	0.06–2.00	3.9–4.7	4.2	4.2	4.5	3.9	4.5	4.3	4.5	*	*
NADP	0.05–0.13	0.04–0.16	6.6–8.4	6.6	†	†	†	†	†	†	*	*
NADP	0.05–0.13	0.16–2.00	12–15	15.0	†	†	†	†	†	†	17.3	19.6

* Velocity too low to be measured.
† Change in velocity between 0.16 mM G-6-P or 0.05 mM NADP concentration and theoretical infinity concentration (intercept of 1/v) was too small to be determined with the enzyme concentration employed. However, a higher Michaelis constant (K_m) above 0.16 mM G-6-P or 0.05 mM NADP concentrations is ruled out.
‡ Data obtained with only the lowest concentration of G-6-P employed in this study; it is significant, however, as it is in close agreement with data obtained by other workers using fluorimetric assays for work under low substrate concentrations (13).

L.B., where only the enzyme of lower specific activity was obtained. The comparisons of kinetic parameters between the enzyme of different specific activities would thus seem valid in this case. If the variation in K_m values were due to the presence of a contaminating competitive type inhibitor in the enzyme preparation, one would expect to obtain different K_m values when different concentrations of enzyme were employed. In no case was significant variation observed when different enzyme concentrations were employed for K_m determinations on G-6-PD from an individual subject.

Effects of pH and buffers on reaction velocity. The effect of different concentrations (0.1, 0.01, 0.001 mole per L) of Tris, glycine, sodium phosphate, and potassium phosphate buffers at pH 7.0, 8.0, and 9.0 on the catalytic activity of G-6-PD was studied. At pH 9.0, G-6-PD from normal subjects and deficient subject L.B. gave higher catalytic activity with Tris buffer than with glycine buffer. The G-6-PD from the remaining deficient subjects had higher catalytic activity with glycine rather than with Tris, suggesting a charge difference between the two groups of enzyme. In every enzyme preparation, increasing the concen-

tration of the Tris or glycine buffer from 0.001 mole per L to 0.1 mole per L stimulated the reaction velocity. However, such an increase in the concentration of the phosphate buffers decreased the velocity of the reaction, indicating inhibition by phosphate ion.

Substrate specificity. The K_m values for substrates other than G-6-P and NADP are presented in Table IV. Normal subjects gave a single K_m value for galactose-6-phosphate (gal-6-P) and two K_m values for 2-d–G-6-P. Deficient subject L.B. showed normal K_m values for both substrates. Deficient subject J.P. had the same gal-6-P K_m as the normal, but only the higher 2-d–G-6-P K_m. The remaining deficient subjects showed no activity towards gal-6-P at the 25° C temperature employed and only the lower normal K_m value for 2-d–G-6-P. At 50° C they demonstrated slight catalytic activity towards gal-6-P, but no K_m values could be determined. The CNSHA subjects gave a high K_m value for gal-6-P and only the higher normal K_m value for 2-d–G-6-P. No activity towards glucosamine-6-phosphate was observed with G-6-PD from any of the subjects.

Electrophoresis. The electrophoretic mobilities of the normal and deficient enzyme are shown in

TABLE IV

Substrate specificity of normal and deficient erythrocyte G-6-PD

		Deficient Negroes							CNSHA	
Substrate	Normal	L.B.	F.H.	H.R.	E.E.	B.C.	N.M.	J.P.	H.E.	M.M.
		$K_m \times 10^6$*								
Gal-6-P†	2.0–2.5	2.5	‡	‡	‡	‡	‡	2.0	2.6	3.1
2-d–G-6-P†	0.65–0.71	0.71	0.67	0.69	0.71	0.71	0.65		2.3	2.3
	2.0–2.5	2.5						2.5		

* The average of two or more determinations.
† Gal-6-P = galactose-6-phosphate; 2-d–G-6-P = 2-deoxyglucose-6-phosphate.
‡ No K_m could be determined at 25° C as there was no detectable catalytic activity. Slight activity was observed at 50° C, but no K_m values were determined.
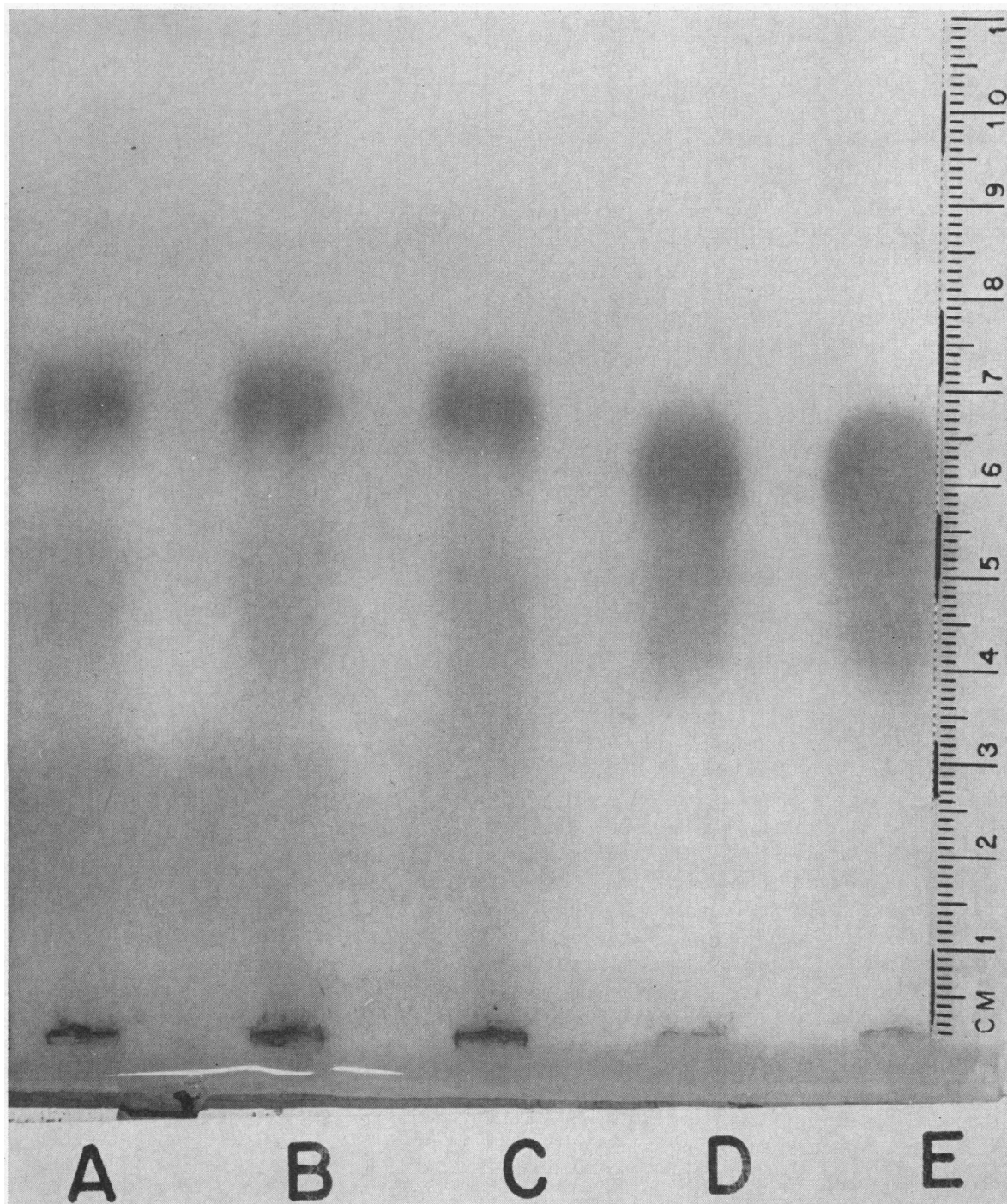
FIG. 4. VERTICAL STARCH-GEL ELECTROPHORESIS OF CRUDE HEMOLYSATES FROM NORMAL AND DEFICIENT SUBJECTS. *A*) B.C., deficient type II. *B*) J.P., deficient type III. *C*) M.M., congenital nonspherocytic hemolytic anemia, type IV. *D*) L.B., deficient type I. *E*) Normal. The origin lies near the bottom of the Figure. The anode is at the top and the cathode at the bottom of the Figure. Hemoglobin forms the lighter areas below the darker G-6-PD bands.

TABLE V

Classification and characteristics of deficient erythrocyte G-6-PD

	Normal enzyme	Deficient enzyme type I (L.B.)	Deficient enzyme type II (F.H., H.R., E.E., B.C., N.M.)	Deficient enzyme type III (J.P.)	Deficient enzyme type IV (H.E., M.M.)
E_a, 20°-30° C, kcal/mole	10	10	5	5	10
E_a, 40°-50° C, kcal/mole	5	5	10	5	
$K_m \times 10^6$					
G-6-P	30–50	30–50	30–50	30–50	
G-6-P	70–88	70–88			90
NADP	1.7–2.3	1.7–2.3	1.7–2.3	1.7–2.3	
NADP	3.9–4.7	3.9–4.7	3.9–4.7	3.9–4.7	
NADP	6.6–8.4	6.6–8.4			
NADP	12–15	12–15			15–20
$K_m \times 10^3$					
Gal-6-P	2.5	2.5	No activity	2.0	2.5–3.1
2-d-G-6-P	0.71	0.71	0.71		
2-d-G-6-P	2.50	2.50		2.50	2.3
Electrophoresis	Slow (B⁺)	Slow (B⁻)	Fast (A⁻)	Fast (A⁻)	Fast (A⁻)
Ratio of velocities in Tris/glycine (0.1 M each)	>1.0	>1.0	<1.0	<1.0	<1.0

Figure 4. Normal and deficient type I (L.B.) moves identically to the slower B band (channels E and D, respectively). Deficient type II (F.H., H.R., E.E., B.C., and N.M.), deficient type III (J.P.), and CNSHA (H.E. and M.M.) move faster than the normal and deficient type I, corresponding to the faster A band (channels A, B, and C, respectively). Similar electrophoretic mobilities were obtained with the purified enzyme. Deficient types II and III and our CNSHA subjects cannot be distinguished from each other by the electrophoretic procedures employed. The results suggest that the enzymes may be divided into two groups due to a charge difference on the enzyme molecule and confirm that enzyme deficiency may occur with G-6-PD of slow or fast electrophoretic mobility.

Discussion

The parameters of G-6-PD investigated suggest the existence of four biochemically distinguishable enzyme types in the deficient human subjects employed in this study The characteristics of each group are summarized in Table V. Deficient enzyme types I and III comprise only single deficient subjects, L.B. and J.P., respectively. Deficient enzyme type II is composed of five deficient subjects, F.H., H.R., E.E., B.C., and N.M. The CNSHA subjects employed comprise deficient enzyme type IV, which appears to be only one of several qualitatively different G-6-PD enzyme types reported for CNSHA subjects (19).

The determination of two K_m values for G-6-P with normal G-6-PD suggests the presence of two catalytic sites on the enzyme (23, 24). The implications of two catalytic sites with respect to supporting evidence and reaction mechanisms will be discussed elsewhere (25). However, the similarity of K_m values that could be determined with each of the normal and deficient enzymes suggests that the qualitative differences observed in this investigation may be due to the absence or lack of activity at one or the other of the two catalytic sites.

Qualitative differences in G-6-PD based on kinetic data have been reported only for CNSHA subjects and Caucasian drug-sensitive males (6, 9, 11, 18, 19). The G-6-PD from our CNSHA subjects is characterized by a fast electrophoretic pattern, a higher than normal K_m value for G-6-P, and a marked thermal instability. Thus the enzyme deficiency of our CNSHA subjects is apparently also due to a qualitative enzyme abnormality.

The detailed characterization of deficient enzyme type I demonstrates that it is identical with the normal enzyme in all of the parameters investigated. Consequently, the deficiency observed may be attributed to lower enzyme concentration and not to a lower reaction rate due to enzyme abnormality. Therefore in type I we have a quantitative difference in G-6-PD between the normal and deficient subject.

Deficient enzyme types II, III, and IV may be

readily distinguished from the normal enzyme with respect to electrophoretic mobility, K_m values, and activation energies. While each of these three types is qualitatively different from the normal enzyme, they are also qualitatively different from each other.

Sex-linked qualitative and quantitative differences between the enzymes of normal and drug-sensitive patients have been based by other workers on electrophoretic mobility or activity in the hemolysate. The latter gives no indication of whether the deficiency is due to enzyme concentration or enzyme rate; the former is not reliable because electrophoretic mobility has no absolute relationship to G-6-PD deficiency (6, 7, 10, 11).

The failure of other investigators to detect the qualitative differences reported here for drug-sensitive Negro subjects requires evaluation. It would seem most reasonable to attribute the failure to detect qualitative enzyme differences in drug-sensitive Negro subjects to the more limited characterizations employed by other investigators. If only the lower G-6-P concentration was employed in K_m determinations, the same K_m values would be obtained for normal and deficient types I, II, and III. Generally, in such characterizations, only the lower K_m values for G-6-P, NADP, and 2-d–G-6-P are reported (26, 7), although higher K_m values have been reported for the normal enzyme and some CNSHA subjects (9, 11). Although deficient enzyme types II and III can be distinguished electrophoretically, the quantitative deficiency, deficient enzyme type I, is indistinguishable from the normal enzyme.

Our electrophoretic data obtained from normal and nonanemic G-6-PD-deficient Negro males are in essential accord with those of Boyer, Porter, and Weilbacher (16) and Kirkman and Hendrickson (21). It would appear that the normal and type I enzyme is comparable to electrophoretic type B, and types II and III are comparable to Type A. Porter and his colleagues (6) in their recent review of the variations of G-6-PD in different populations have reported that, with one exception, Negro enzyme-deficient subjects show a fast electrophoretic component. The enzyme characteristics of the G-6-PD-deficient Negro who showed the slow band are not available in Porter's report (6). We would predict that this individual's G-6-PD would show the characteristics of our type I.

More recently, Kirkman, McCurdy, and Naiman (18) have summarized the published data on patients with functionally abnormal G-6-PD. Although several variants have been found in the Caucasians, all those seen in Negroes have been classified as electrophoretic type A⁻, with the exception cited by Porter and associates (6).

Porter and associates (6) have stated that there appears to be extensive genetic heterogeneity of G-6-PD among subjects with the uncommon forms of enzyme deficiency. The electrophoretic mobility of the G-6-PD obtained from the Italian family named Barbieri reported by Marks, Banks, and Gross (11) was faster than normal type B and also faster than type A. Although both parents of one of our Caucasian CNSHA patients and the mother of the other have an Italian background, their G-6-PD electrophoretic mobility is similar to type A. The family of Marks and colleagues, however, is atypical in that the red cell G-6-PD level is about one-half of normal, in contrast to the extremely low levels in our two patients and those reported by others for Caucasians with CNSHA. Kirkman and associates have reported patients with CNSHA with G-6-PD electrophoretic mobility similar to normal Caucasians, type B (18, 19). The patient designated "Eysson" by Boyer and colleagues (16) showed a slower mobility than type B. Obviously, comparative studies of these more unusual deficient patients should be carried out.

Summary

Glucose-6-phosphate dehydrogenase from erythrocytes of normal and enzyme-deficient subjects has been characterized in detail. The comparison of a number of parameters from drug-sensitive, nonanemic, Negro males indicates the existence of three qualitatively different enzyme types. Type I is indistinguishable from the normal Negro enzyme, having a slow type B electrophoretic pattern and identical activation energies and kinetic constants. Types II and III give a fast type A electrophoretic pattern and abnormal activation energies and kinetic constants. However, although types II and III could not be distinguished from each other electrophoretically, they could be separately characterized on the basis of their activation energies, substrate specificity, and kinetic constants for 2-deoxyglucose-6-phosphate. Par-

tially purified glucose-6-phosphate dehydrogenase from Caucasian male congenital nonspherocytic hemolytic anemic patients formed a separate group, type IV, with a fast type A electrophoretic pattern, a modified activation energy, and modified kinetic constants. Type IV could be readily distinguished from types I, II, and III by its K_m values and thermal stability.

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