Genetic Studies on Southeastern Bantu of Mozambique. I. Variants of Glucose-6-Phosphate Dehydrogenase

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INTRODUCTION

Studies of glucose-6-phosphate dehydrogenase (G6PD) polymorphism in African Bantu have primarily detected variants associated with G6PD deficiency. However, the polymorphism of this enzyme is more extensive in Negroes, since a common G6PD allele (Gd^A) and other sporadic mutants are found in Negro populations by electrophoretic techniques. The studies described in the present report were designed to reveal the maximal extent of variation at the G6PD locus, within the limits of the present screening methodology. The subjects studied belong to the East African Bantu inhabiting southern Mozambique.

The Bantu tribes in Mozambique can be allocated to four major clusters (Murdock 1959). From north to south, they are the Yao, Maravi, Shona, and Shangana-Thonga (fig. 1). The ramifications of these clusters extend beyond the political and geographical borders of Mozambique into the neighboring countries. No significant intermixing occurs among the clusters not only because they are tribally endogamic, but because two major rivers constitute natural barriers between them. Thus, the Maravi and Yao occupy the territory north of the Zambesi River; the Shangana-Thonga, south of the Save River; and the Shona, between the two rivers. The Yao and Maravi tribes are the eastern representatives of the central Bantu group. The Shona and Shangana-Thonga are southeastern Bantu. Tribes belonging to the last group were examined in this study.

The affiliation of Shona and Shangana-Thonga to early migratory flows is uncertain. Each of the clusters numbers more than one million people and is culturally quite homogeneous. However, in the recent past both clusters have received considerable increments from the southernmost Bantu group, the Nguni. The Nguni invaded and established a conquest state in Shona territory during the early nineteenth century (Murdock 1959). Arabic influence is found north of Zambesi, particularly in coastal tribes, but this influence is much less significant in the Shona and

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Shangana-Thonga clusters. The southeastern Bantu tribes probably occupied their territory four or five centuries before the arrival of the Portuguese discoverers in 1554.

The Bantu tribes surveyed in the present report were the Ronga, Thonga, Shangana, and Chopi. Although all are grouped in the Shangana-Thonga cluster, some authors consider the Chopi affiliated to the Shona cluster (Velez-Grilo 1960). It has also been suggested that the tribe of Shangana represents a drift from the Nguni

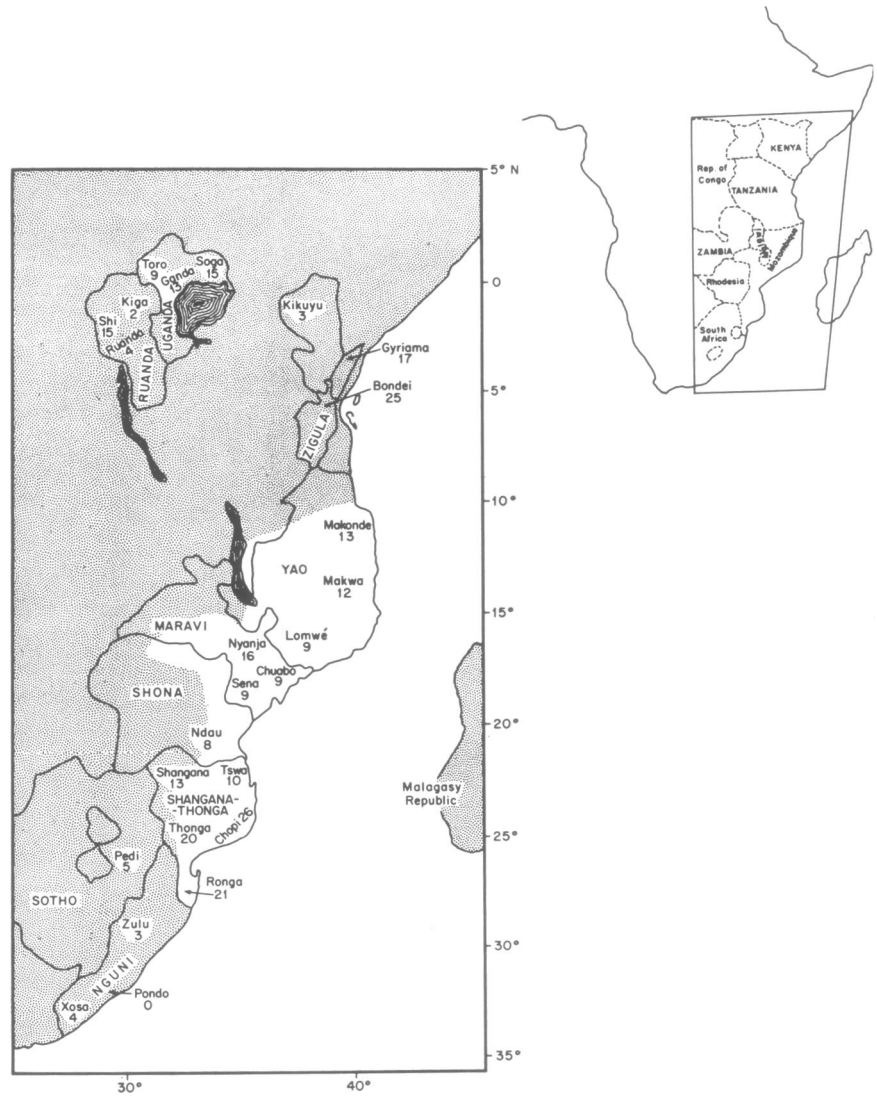

FIG. 1.—Map of southeast Africa showing the prevalences of G6PD deficiency (%) in Mozambique (unshaded area) and neighboring territories. The demarked areas represent the approximate limits of the major Bantu provinces where the G6PD deficiency has been surveyed.

into the Thonga cluster. The Chopi and Thonga seem to be the most primitive of the tribes living in southeastern Africa (Simões Alberto 1962).

MATERIALS AND METHODS

Two surveys have been conducted on this population. In the first (sample A), 315 healthy, male Negro army recruits were screened for G6PD polymorphisms and other genetic markers discussed in the report of Giblett and co-workers (in preparation). In the second (sample B), 450 male Negro blood donors to the blood bank of the Hospital Central Miguel Bombarda (Lourenzo Marques) were screened for G6PD variation only. Blood samples were obtained by venipuncture and collected in acid-citrate-dextrose solution (NIH formula B), stored at 4° C, and studied within one week after blood withdrawal. For the purpose of characterizing G6PD variants, new samples from the propositi and their families were collected.

Screening Techniques

The screening techniques for G6PD variation included the brilliant cresyl blue (BCB) decolorization test (Motulsky and Campbell-Kraut 1961) and electrophoresis of red blood cell hemolysates. The electrophoretic studies of sample A were done using tris-EDTA-borate (TEB) buffer system at *p*H 8.6 (Porter et al. 1964). Studies of sample B used starch gel electrophoresis with tris-HCl buffer at *p*H 8.8. (Kirkman and Hendrickson 1963) and phosphate buffer at *p*H 6.8 (Mathai et al. 1966) and electrophoresis in cellulose acetate gel with tris-citrate buffer *p*H 7.5 (Ratazzi, Bernini et al. 1967). The TEB buffer system described for sample A was also used.

Characterization of G6PD Variants

Partially purified G6PD was obtained from the red cells as described by Motulsky and Yoshida (1969). The activity of 6PGD was always assayed after dialysis and found to be less than 1% of G6PD activity. The electrophoretic migration of the partially purified enzyme was studied with the screening techniques described. The kinetic studies included determination of Michaelis constants for G6P and NADP, relative utilization of 2-deoxy-d-glucose-6-phosphate and deamino-NADP (dNAPD) (Motulsky and Yoshida 1969), *p*H dependence (Kirkman, Rosenthal et al. 1964), and heat stability (Kirkman, Schnettini, and Pickard 1964). Samples from persons with normal electrophoretic patterns and G6PD activity were used as controls in each experiment.

As an additional criterion for G6PD characterization, inhibition and inactivation of the enzyme by N-ethyl-maleimide (NEM) and hydroxymercuribenzoate (HMB) were measured (Luzzatto and Afolayan 1968). Preparations from the variant enzymes together with appropriate controls were dialysed overnight at 4° C against tris-buffer 0.05 M, *p*H 8.0, which contained EDTA 0.027 M and NADP 10^{-4} M. The enzyme activities were then adjusted to 0.05 IU/ml with the same buffer. For inhibition studies, the final concentrations of NEM and HMB were in the range of 4×10^{-4} M to 4×10^{-3} M. The inactivation was measured after a preincubation of the enzyme preparation (30 minutes for HMB and 60 minutes for NEM) at 25° C;

final concentrations of HMB and NEM in the incubation mixture ranged from 10^{-2} to 10^{-6} M.

RESULTS

The Common G6PD Polymorphisms

The screening of the two samples of males by the BCB test gave a G6PD deficiency of 20% in sample A and 18% in sample B. Simultaneous electrophoretic screening indicated that none of these deficient samples were of the Mediterranean (G6PD B$^-$) variety. Most of the G6PD-deficient samples were the common Negro type of G6PD deficiency (GP6D A$^-$). In one case G6PD deficiency was due to a new structural G6PD mutant.

TABLE 1

G6PD POLYMORPHISMS IN SOUTHEASTERN BANTU

TRIBE	NO. MALES TESTED	GENE FREQUENCIES			RARE VARIANTS
		Gd^B	Gd^A	Gd^{A-}	
Sample A:					
Ronga	60	.72	.13	.15	1*
Shangana	102	.61	.17	.22	0
Thonga	61	.72	.12	.16	1*
Chopi	62	.48	.24	.28	1*
Sample B	450	.67	.15	.18	6†
Total	735	.....	.....	.....	9
Averages	.....	.63	.17	.19	0.01

* Not characterized variants, electrophoretically similar to G6PD Lourenzo Marques.

† Characterized variants: G6PD Chibuto in Shangana; G6PD Lourenzo Marques in Thonga; G6PD Inhambane in Thonga; G6PD Manjacaze in Chopi. For two cases with G6PD Lourenzo Marques, tribal origin unknown.

The findings of the electrophoretic screening are presented in table 1. For sample A, detailed information of tribal origin was available; in sample B, the tracing of tribal origin was not possible in every case. However, most individuals in sample B are city dwellers to which the Ronga and Shangana tribes are the main contributors.

The New G6PD Variants

Three specimens in sample A had electrophoretic migration intermediate to that of G6PD B and G6PD A. Characterization of these variants was not possible. In sample B, six showed an abnormal electrophoretic mobility in at least two of the electrophoretic systems used. The carriers of these variants were clinically and hematologically normal blood donors. Findings of the enzymatic studies are presented in table 2. For the designation of the variants, the origin of the propositi was used (World Health Organization 1967).

The variant G6PD Chibuto was observed in a Shangana. It is a variant with mild deficiency (20% of G6PD B controls) and electrophoretic mobility slower than G6PD A. The difference is more evident when phosphate buffer at *p*H 6.8 (fig. 2) or cellulose

TABLE 2

ELECTROPHORETIC AND KINETIC CHARACTERIZATION OF THE NEW G6PD VARIANTS*

	G6PD Inhambane	G6PD Chibuto	G6PD Lourenzo Marques	G6PD Manjacaze	G6PD B	G6PD A	G6PD A⁻
Activity in red blood cells†	100	20	100	100	100	80–100	8–20
Electrophoretic mobility:‡							
*p*H 8.6	112	108	106	90	100	110	110
*p*H 6.8	115	109	106	90	100	112	112
K_m (μM):							
G6P	38	30	66	141	40–75	40–75	40–75
NADP	4.7	8.2	4.3	3.8	2.9–6.0	2.9–6.0	2.9–6.0
Substrate analogues:§							
2dG6P	<4	<4	<4	<4	4–5 or less	4–5 or less	4–5 or less
dNADP	50	61	55	52	50–60	50–60	50–60
*p*H curve	Slightly biphasic	Truncate	Truncate	Truncate	Truncate	Truncate	Truncate
Heat stability	Normal	Slightly increased	Normal	Normal	Normal	Normal	Normal

* Abbreviations used: G6P = D-glucose-6-phosphate; NADP = nicotine-adenine-diphosphonucleotide; 2dG6P = 2-deoxy D-glucose-6-phosphate; d-NADP = deamino-NADP.

† Erythrocyte G6PD activity expressed as percentage of mean normal values.

‡ Electrophoretic mobility expressed as percentage relative to G6PD B in the same buffer.

§ Utilization of 2dG6P and d-NADP as percentage of utilization of G6P and NADP respectively

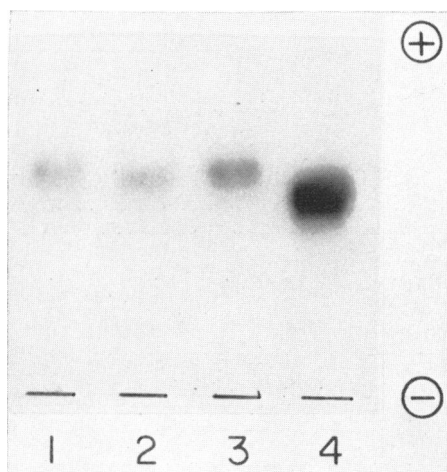

FIG. 2.—Horizontal starch gel electrophoresis in phosphate buffer system; *p*H 6.8; gradient 6 v/cm per six hours at 4°C. 1 = G6PD A; 2 = G6PD Chibuto; 3 = G6PD A + G6PD Chibuto; 4 = G6PD B.

acetate gel are used; in TEB buffer system, G6PD Chibuto moves closer to G6PD A (table 2). In addition to electrophoretic mobility, G6PD Chibuto differs from G6PD A$^-$ by slightly increased heat stability, relatively increased resistance to inhibition and inactivation with NEM and HMB (fig. 3), and a higher K_m for NADP. G6PD Chibuto differs* from G6PD Kephalonia and G6PD Attica (Rattazzi et al. 1969) in K_m for NADP and in that the latter variants are not distinct from G6PD A$^-$ on starch gel electrophoresis in the TEB buffer system. The mobility in TEB and phosphate buffer systems, the reduced heat stability, and the biphasic *p*H dependence curve distinguish G6PD Canton from G6PD Chibuto.

The variant G6PD Lourenzo Marques was observed in a Thonga. This mutant is characterized by normal activity in red blood cells and electrophoretic migration intermediate between G6PD A and G6PD B (fig. 4). Samples from three unrelated

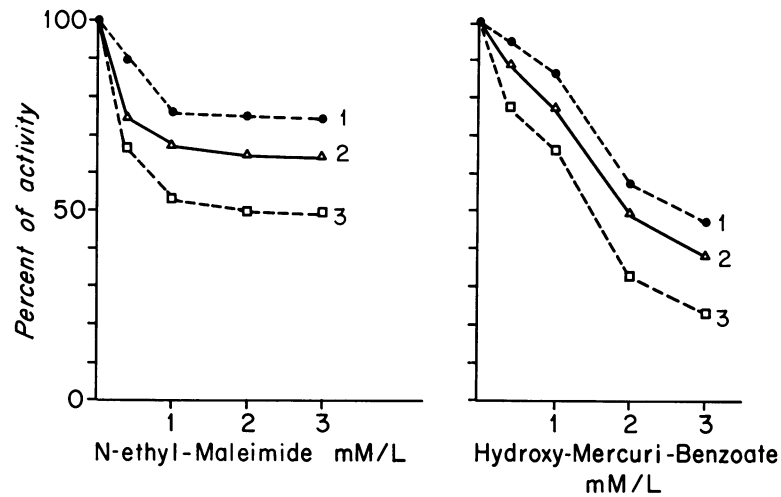

FIG. 3.—Comparative inhibition of G6PD Chibuto and G6PD A$^-$ by N-ethyl-maleimide and hydroxymercuribenzoate. Activity expressed as percentage of control without inhibitor. 1 = G6PD Chibuto; 2 = G6PD Chibuto plus G6PD A$^-$; 3 = G6PD A$^-$.

blood donors exhibited this electrophoretic mobility. A mixture of these three samples did not resolve into distinctive bands, so it is possible that these carriers possessed G6PD Lourenzo Marques. The kinetic parameters of G6PD Lourenzo Marques are within the range of normal G6PD. The variant G6PD Lourenzo Marques is very similar to G6PD King County (Yoshida et al. in preparation) and G6PD Levadia (Stamatoyannopoulos et al. in preparation). Distinction between these variants was achieved through direct electrophoretic comparison in starch gel, using phosphate buffer *p*H 6.8 and high-voltage electrophoresis (8 v/centimeter for six hours). Under these conditions, G6PD Lourenzo Marques moves faster than G6PD King County, and slower than G6PD Levadia.

* Variants with electrophoretic mobility similar to the variants of the present study but without any kinetic characterization available (G6PD "Joliet," G6PD "Lagos," G6PD "São Paulo 2") are not included in these comparisons.

The variant G6PD Manjacaze was found in a Chopi. This variant has normal activity in red blood cells and a mobility slower than G6PD B in all buffer systems used (fig. 5). It has significantly increased K_m for G6P, while its affinity for NADP is normal. Inhibition and inactivation with sulfhydryl reagents did not reveal any significant difference between G6PD Manjacaze and G6PD B. The variant G6PD Manjacaze should be compared to the several mutants characterized by an electrophoretic mobility slower than G6PD B and normal enzyme activity in red blood cells (Motulsky and Yoshida 1969). With the exception of G6PD Ita-Bale (Luzzatto and Afolayan 1968), G6PD Manjacaze can be distinguished from all these variants on the basis of K_m for G6P. The electrophoretic migration of G6PD Ita-Bale

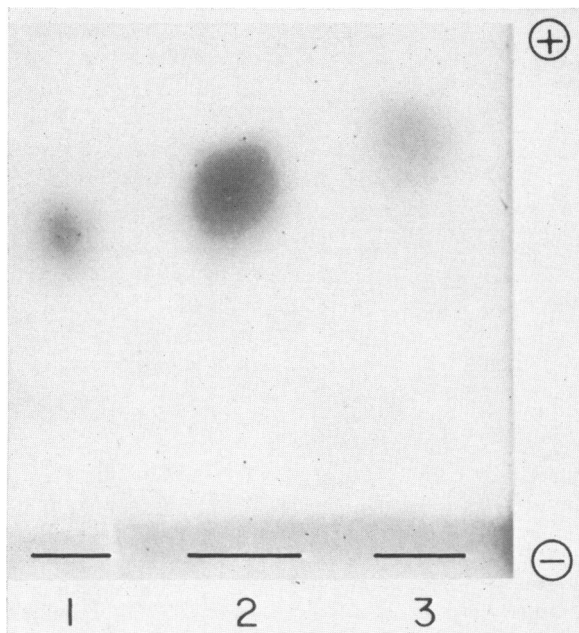

FIG. 4.—Horizontal starch gel electrophoresis in tris-buffer system; *p*H 8.8; gradient 4 v/centimeter for 16 hours at 4°C. 1 = G6PD B; 2 = G6PD Lourenzo Marques; 3 = G6PD A.

(30% of concurrent control G6PD B) is the feature which distinguishes this variant from G6PD Manjacaze.

The mutant G6PD Inhambane (observed in a Thonga) is characterized by normal G6PD activity in red blood cells. On electrophoresis it moves faster than G6PD A (fig. 6) and this is accentuated when a phosphate buffer system at *p*H 6.8 is used. The kinetic parameters of this enzyme do not differ from normal. Both G6PD Inhambane and G6PD A have similar susceptibilities to inhibition and inactivation by sulfhydryl compounds. No other variants with the characteristics of G6PD Inhambane have been reported. The variant G6PD "Andra Pradesh" originally reported with an electrophoretic migration of 120% of normal (Rattazzi and Lenzerini 1967), was subsequently found to have a mobility close to G6PD B in cellulose acetate gel and to G6PD A in a more alkaline *p*H (Rattazzi, unpublished).

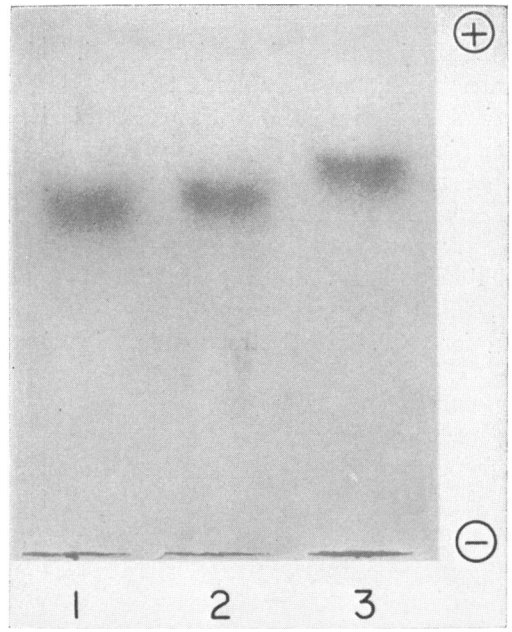

FIG. 5.—Horizontal starch gel electrophoresis in TEB buffer system; *p*H 8.6; gradient 6 v/centimeter for six hours at 4°C. 1 = G6PD Manjacaze; 2 = G6PD B; 3 = G6PD A.

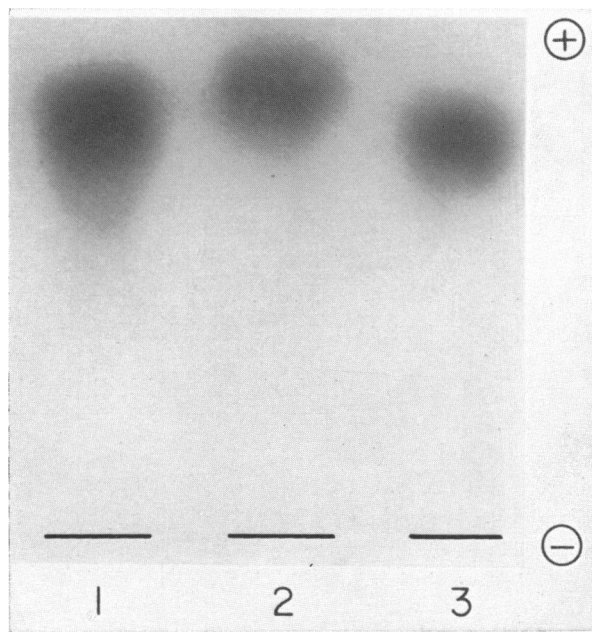

FIG. 6.—Horizontal starch gel electrophoresis in phosphate buffer system; *p*H 6.8. 1 = G6PD A; 2 = G6PD Inhambane; 3 = G6PD B.

Family studies indicated transmission of these variants was compatible with sex-linked patterns of inheritance.

DISCUSSION

The polymorphism of G6PD in the southeastern Bantu is within expectations for Negro populations. Both Gd^A and Gd^{A-} were frequent in the tribes studied, although the gene associated with enzyme deficiency in red blood cells (Gd^{A-}) was more frequent than the Gd^A allele. In most electrophoretic G6PD studies in Negroes, the opposite situation seems to prevail (table 3). However, only two African Bantu populations are available for comparison. In Nigerian Yoruba the frequencies of Gd^A and Gd^{A-} are identical (Porter et al. 1964), while in the South African Bantu tribes

TABLE 3

PREVALENCE OF Gd^B, Gd^A, AND Gd^{A-} FREQUENCIES IN NEGROID POPULATIONS*

POPULATION ORIGIN	NO. MALES TESTED	GENE FREQUENCIES				RATIO Gd^{A-}/Gd^A	REFERENCE
		Gd^B	Gd^A	Gd^{A-}	Gd^A and Gd^{A-}		
American	311	.66	.16	.18	.....	1.13	Porter et al. 1964
	135	.65	.23	.12	.....	0.52	Kirkman and Hendrickson 1963
	1941	.71	.18	.11	.....	0.61	Yoshida et al. 1967
Brazil:							
Northeast	1783	.87	.06	.07	.....	1.17	Nance 1964
Negroes (São Paulo)	48	.86	?	?	.14	.....	Saldanha et al. 1969
Africa:							
Nigerians (Yoruba)	141	.56	.22	.22	.....	1.00	Porter et al. 1964
Bantu	90	.82	.12	.06	.....	0.50	Balinsky and Jenkins 1966
Bushmen	35	.95	.03	.02	.....	0.67	Balinsky and Jenkins 1966
Negroes	150	.85	?	?	.15	.....	Gordon et al. 1966
Negroid (Surinam)	217	.79	?	?	.21	.....	Smink and Prins 1965
Mozambique SE Bantu	315	.64	.17	.19	.....	1.12	Present study

* Studies based on less than 30 males examined are not included in the table.

Gd^A is nearly twice as frequent as Gd^{A-} (Balinsky and Jenkins 1966). Since the dynamics of Gd^{A-} and Gd^A genes in African populations are largely unknown, the findings in the southeastern Bantu are difficult to interpret. The observed high Gd^{A-}/Gd^A ratio in the Shangana-Thonga may be related to tribal isolation and drift. It could also be due to a greater selective advantage of Gd^{A-} carriers in the southeast African environment, or to the lesser disadvantage of the Gd^{A-} hemizygotes in that environment.

Among the 735 southeastern Bantu of this study, nine had a G6PD electrophoretically different from G6PD B or G6PD A.

The detailed electrophoretic and kinetic characterization of the enzyme in six cases revealed that they possessed four variants different from the already described G6PD mutants. Within the limitations of the present methodology, these four G6PDs should be considered as new variants. However, final judgment is not possible until the structure of these variants is studied by G6PD fingerprinting techniques and

TABLE 4

G6PD DEFICIENCY FREQUENCIES IN AFRICAN BANTU POPULATIONS*

Group and Cluster	Tribe	No. Males Tested	Gene Frequencies	Country	Reference
Central Bantu:					
Kwango	Yaka	628	.234	Congo	Motulsky et al. 1966
Kimbundu	Bangala	140	.286	Angola	Santos-David 1965
	Shinje	96	.167	Angola	Santos-David 1965
Maravi	Chuabo	248	.089	Mozambique	Reys et al. 1965
	Nhungwe	214	.070	Mozambique	Reys et al. 1965
	Sena	297	.091	Mozambique	Reys et al. 1965
	Nyanja	378	.156	Mozambique	Reys et al. 1965
Yao	Makonde	382	.131	Mozambique	Reys et al. 1965
	Makwa	980	.120	Mozambique	Reys et al. 1965
	Lomwé	53	.094	Mozambique	Reys et al. 1965
Interlacustrine Bantu:					
Ruanda	Shi	92	.152	Congo	Motulsky et al. 1966
	Ruanda†	231	.043	Congo & Uganda	Motulsky et al. 1966; Luttrell and Lea 1965
	Nkole†	73	.027	Uganda	Luttrell and Lea 1965; Knight and Robertson 1965
	Kiga†	65	.015	Uganda	Luttrell and Lea 1965; Knight and Robertson 1965
Uganda	Soga†	61	.148	Uganda	Luttrell and Lea 1965; Knight and Robertson 1965; Robertson 1961
	Ganda†	503	.129	Uganda	Luttrell and Lea 1965; Knight and Robertson 1965; Allison 1960
	Toro†	43	.089	Uganda	Luttrell and Lea 1965; Knight and Robertson 1965
NE Coastal Bantu:					
Nika	Gyriama	101	.168	Tanzania	Allison 1960
Zigula	Bondei†	545	.253	Tanzania	Allison 1960; Allison and Clyde 1961
Kenya Highland Bantu	Kikuyu	70	.029	Kenya	Allison 1960
Southern Bantu:					
Sotho	Kgalagadi	49	.000	S. Africa	Jenkins et al. 1968
	Pedi†	56	.054	S. Africa	Bernstein 1963; Charlton and Bothwell 1961
Nguni	Xosa†	227	.035	S. Africa	Bernstein 1963; Charlton and Bothwell 1961
	Pondo	44	.000	S. Africa	Bernstein 1963
	Zulu†	189	.032	S. Africa	Bernstein 1963; Charlton and Bothwell 1961
	Msutu†	159	.038	S. Africa	Bernstein 1963; Charlton and Bothwell 1961
Shangana-Thonga:					
Shona	Ndau	101	.079	Mozambique	Manso 1966
Shangana	Shangana†	514	.130	S. Africa & Mozambique	Charlton and Bothwell 1961; Reys et al. 1965‡
Thonga	Tswa	62	.097	Mozambique	Reys et al. 1965
	Thonga†	162	.198	Mozambique	Reys et al. 1965‡
	Ronga†	204	.206	Mozambique	Reys et al. 1965‡
	Chopi†	149	.262	Mozambique	Reys et al. 1965‡

* Tribes not studied or with frequencies based on less than 30 males examined are not included in the table.

† Combined data from independent studies.

‡ Data from present study included.

amino acid analysis of the tryptic peptides. The simultaneous application of three buffer systems was important in the recognition of the variants, since variants which could have been missed in one buffer system (e.g., in tris-buffer G6PD Chibuto and G6PD Inhambane) were immediately recognized in the parallel electrophoretic examination by another buffer system (e.g., G6PD Chibuto and G6PD Inhambane in phosphate buffer). Thus the combination of techniques allowed the detection of approximately one variant per 100 males studied. This high degree of diversity of the G6PD mutants is not unique. A similar degree of G6PD heterogeneity has been also observed in Greeks (Stamatoyannopoulos et al. in preparation). Eight of the Mozambique variants had an electrophoretic migration faster than the common G6PD B. It is possible that in previous electrophoretic screening of Negro populations, such enzymes have been categorized as G6PD A or G6PD A^- on the basis of staining intensity. The findings of the present survey indicate that such designations should be avoided unless appropriate controls are present in the same gel for comparison.

While information on electrophoretic G6PD polymorphism in Bantu tribes is scanty, data on the frequencies of deficient mutants in several tribes are available (table 4). As would be expected from the widespread environments of the Bantu, a broad range in the frequencies of deficient mutants has been observed. Such variation in the Gd^- frequencies is usually attributed to corresponding variation in the degree of malaria pressure (Motulsky 1964). Variation in Gd^- frequencies is also present in the Bantu tribes of Mozambique and a definite pattern has been observed in one previous study (Reys et al. 1965). High Gd^- frequencies are observed in the Yao and Maravi living north of Zambesi and in the Shangana-Thonga tribes; lower frequencies (fig. 1) in the Shona tribes inhabiting the area between the Zambesi and Save. In the Nguni tribes living in South Africa, the Gd^- prevalence is rather low (Balinsky and Jenkins 1966; Bernstein 1966; Charlton and Bothwell 1961).

In the absence of sound evidence on the origin of tribes and the migration flows, these patterns are difficult to interpret. The controversy regarding the affiliation of the Chopi illustrates the need for additional detailed studies on the genetic structure of these populations. If it were known that the Chopi were related to the Shona as some authors feel (Velez-Grilo 1960), the higher Gd^{A-} frequencies in the Chopi could be interpreted to indicate an increase in fitness of Gd^{A-} carriers in the southern Mozambique environment.

SUMMARY

In a survey of 735 male African southeastern Bantu living in Mozambique, an average of 63% G6PD B, 17% G6PD A, and 19% G6PD A^- was found. Those G6PD electrophoretic mutants other than G6PD A or G6PD A^- had an approximate frequency of 1.2%. Enzyme characterization revealed four probable new variants: G6PD Inhambane, G6PD Chibuto, G6PD Lourenzo Marques, and G6PD Manjacaze.

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