Associations Between Red Cell Glucose-6-Phosphate Dehydrogenase Variants and Vascular Diseases

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THIS SEARCH FOR possible associations between red cell glucose-6-phosphate dehydrogenase (G6PD) variants and vascular diseases was begun primarily to see if coronary artery disease is less frequent among Negro men with the two common red cell G6PD variants, type A (the electrophoretic variant with essentially normal activity) and type A(−), than among Negro men with type B G6PD.

The possibility of a negative association between coronary artery disease and the G6PD variants found in about 35% of Negro males (Boyer *et al.*, 1962; Kirkman and Hendrickson, 1963) is raised by the lower frequency of coronary artery disease almost consistently reported for Negroes than for Caucasians both in the United States and in Africa (reviewed by Phillips and Burch, 1960) and the lower frequency of that disease among the non-Ashkenazic compared with the Ashkenazic Jews in Israel (Toor *et al.*, 1960; Kallner, 1962). At least one deficient G6PD variant, different from those of Negro populations, is relatively common among the non-Ashkenazic Jews, whereas occurrence of G6PD variants among the Ashkenazic Jews, as among Northern European Caucasians, is rare (Tarlov *et al.*, 1962).

That a part of this difference in the frequency of coronary artery disease between Negro and Caucasian and between non-Ashkenazic and Ashkenazic Jew is environmental is suggested by the tendency for the difference to disappear as living conditions, including diet and exercise, become more similar for the populations involved (Toor *et al.*, 1960; McDonough *et al.*, 1965). This survey was also prompted, however, by the possibility that a TPNH deficiency secondary to deficient G6PD variants might influence atherosclerosis by limiting those reactions involved in fat and cholesterol metabolism which require TPNH as a coenzyme (Siperstein, 1959).

Received July 11, 1966.

Supported by U. S. Public Health Service research grant HE 04516 from the National Institutes of Health.

Presented in part at the annual meeting of The American Society of Human Genetics, Seattle, Washington, August 25, 1965.

Since the gene or genes which determine structure and activity of G6PD are located on the X chromosome and since the Negro female is often heterozygous for the usual form of G6PD (type B) and one of the variant forms (Boyer *et al.*, 1962; Kirkman and Hendrickson, 1963), this study was limited to males. For several reasons, including a higher frequency of hypertensive disease and some types of idiopathic cardiomyopathy reported for Negro than for Caucasian populations (Phillips and Burch, 1960), hospital records were reviewed for evidence of all diseases.

METHODS

Blood samples were obtained over an 18-month period from 1,382 Negro males over the age of ten years who were patients attending the General Medical and Medical Specialty Out-Patient Clinics of Parkland Memorial Hospital, the teaching hospital of the University of Texas Southwestern Medical School, in Dallas. An additional 91 samples were obtained from an apparently similar population of outpatients at Ben Taub Hospital, Houston, Texas. They are combined with the Dallas patients to make a total of 1,473 Negro male patients over the age of ten years. Samples were collected in especially prepared Vacutainers (Becton, Dickinson, and Company) containing 1.5 ml of ACD Solution A for about 8.5 ml of blood.

To identify red cell G6PD deficiency, the methemoglobin reduction test (Brewer *et al.*, 1962) was run on each sample, positive and questionably positive tests for G6PD deficiency being checked by red cell G6PD assay. The double-substrate method used is described by Long *et al.* (1965). This check was considered necessary because blood was stored in a refrigerator for up to three weeks before tests were run. For differentiation of red cell G6PD types on electrophoresis, the procedure of Kirkman and Hendrickson (1963) was used.

Rh blood types were determined for most samples. The antisera against C, D, E, c, and e were obtained from Ortho Pharmaceutical Corporation. Hemoglobin variants were noted at the time of starch gel electrophoresis. Serum cholesterol values were obtained from the clinic records.

Diagnoses were compiled by the senior author after review and abstract of the complete clinical records for each patient. Diagnostic disagreement between the senior author and the examining physicians was rare. Most records were well written. Since the patients were seen on a medical teaching service, extensive clinical and laboratory studies were usually available in the records. For most clinics, these studies included routine blood pressure determinations on every visit.

Tests for possible association between disease frequencies and red cell G6PD types were performed in three ways. First, for each red cell G6PD type, the number of patients with a given disease was compared with the number having all other diseases. Disease frequency determined on this basis was then tested for heterogeneity among patients with the three G6PD types by chi square test with two degrees of freedom. If evidence for heterogeneity was found, separate tests of disease frequency for those with type A against those with type A(−), with type A against type B, and with

type A(−) against type B were made. Chi square test with one degree of freedom was used.

Second, the attempt was made during review of the records of the patients to determine the disease which caused clinic admission on the day blood was obtained for this study. A primary cause of clinic admission was not always clear for patients with multiple diseases, but it could be estimated for 363 over the age of 30 years with type A and A(−) G6PD combined as a group and for 728 with type B G6PD. For each common disease, those with type A and A(−) G6PD as a group were tested against those with type B G6PD in regard to the number with a given disease as a cause for clinic admission compared with the number of all other patients with that G6PD type for whom clinic admission cause could be determined. Chi square test with one degree of freedom was used.

Third, the number of patients with each of the common diseases and type A, type A(−), and type B G6PD was compared by chi square test (two degrees of freedom) with the number of persons found to have type A, type A(−), and type B G6PD in a survey of 204 presumably healthy, unrelated Negro males, of mean age 26 years, who were selected by a field worker from groups on street corners, in stores, garages, etc. in Austin, Texas. Type A G6PD was found in 52 (25.5%), type A(−) in 25 (12.3%), and type B in 127 (62.2%).

RESULTS

Frequency of G6PD types, R_o blood type, and the hemoglobin variants is given in Table 1 for all of the 1,465 patients over ten years of age, of whom 1,268 were over 30 years of age. Patients were divided into these age groups because some diseases, such as coronary artery disease, were not found in individuals below age 30, whereas other diseases were spread over the age spectrum. Statistical comparisons of disease frequency are accordingly referred to one or the other of these age groups. No patient was younger than 11 years of age. Mean ages for those with the different G6PD types (Table 1) are similar.

Four additional patients not shown in Table 1 had type B(−) G6PD, verified by examination of additional blood samples; four more patients had the rare variant tentatively designated Austin 2 in a previous study (Long *et al.*, 1965). Patients with type B(−) and those with Austin 2 G6PD are not included in the disease comparisons. Of 1,473 patients over the age of ten years, including the eight with the rare variants, 18.3% had type A, 13.4% had type A(−), and 67.8% had type B G6PD. Of 1,385 patients over the age of ten years, 7.0% had hemoglobin AS, 3.0% had hemoglobin AC, and 0.65% had hemoglobin S. R_o blood type was found in 55.2% of 1,369 individuals over the age of ten years.

Distribution is similar among the three G6PD types for R_o blood type ($\chi^2_{[2]} = 0.404$, $P \simeq 0.8$), for hemoglobin AS ($\chi^2_{[2]} = 0.076$, $P \simeq 0.95$), and for hemoglobin AC ($\chi^2_{[2]} = 3.243$, $P \simeq 0.2$).

Table 2 lists those diseases where heterogeneity is found on comparing disease frequencies among patients with the three red cell G6PD types.

TABLE 1. CHARACTERISTICS OF PATIENTS IN THIS STUDY

	Number	Per cent of total	Mean age (years)	R_o blood type	Other Rh types	Hb A	Hb AS	Hb AC	Hb S	Other Hb types
Patients with type A G6PD										
Over age 10	269	18.4	55.1	142	114	229	17	9	2	0
Over age 30	235	18.5	60.1	127	97	202	15	7	1	0
Patients with type A(−) G6PD										
Over age 10	197	13.4	53.1	96	85	158	13	9	0	1
Over age 30	163	12.9	59.8	76	72	130	13	7	0	0
Patients with type A and A(−) G6PD										
Over age 10	466	31.8	54.3	238	199	387	30	18	2	1
Over age 30	398	31.4	60.0	203	169	332	28	14	1	0
Patients with type B G6PD										
Over age 10	999	68.2	54.0	518	414	849	67	24	7	0
Over age 30	870	68.6	58.8	452	357	744	58	21	2	0

TABLE 2. DISEASES OCCURRING WITH DIFFERENT FREQUENCIES IN RELATION TO RED CELL G6PD TYPES

Probability is indicated in parentheses beneath χ^2 values. NS means not statistically significant.

	Number of Patients			Comparisons (χ^2)			
	Type A G6PD	Type A(−) G6PD	Type B G6PD	Heterogeneity (df = 2)	A/A(−) (df = 1)	A/B (df = 1)	A(−)/B (df = 1)
Coronary artery disease	16	11	125	16.88 (< 0.001)	0.0001 (NS)	10.95 (< 0.001)	7.78 (< 0.01)
All other patients*	184	126	586				
Coronary artery disease plus possible coronary artery disease	51	37	284	14.66 (< 0.001)	0.56 (NS)	10.49 (< 0.005)	6.34 (< 0.02)
All other patients	184	126	586				

HCVD	103	68	278	22.53 (< 0.001)	0.424 (NS)	18.02 (< 0.001)	8.43 (< 0.005)
All other patients†	89	68	477				
HCVD plus possible HCVD	146	95	393	26.41 (< 0.001)	0.596 (NS)	21.29 (< 0.001)	9.47 (< 0.005)
All other patients	89	68	477				
Idiopathic cardiomyopathy	9	5	4	18.37 (< 0.001)	0.255 (NS)	18.12 (< 0.001)	10.07 (< 0.005)
All other patients	260	192	995				
Varicose veins plus hemorrhoids	7	12	15	16.82 (< 0.001)	4.07 (< 0.05)	1.49 (NS)	17.14 (< 0.001)
All other patients	228	151	855				
Thrombophlebitis	11	4	12	9.76 (< 0.01)	1.32 (NS)	9.90 (< 0.005)	1.04 (NS)
All other patients	224	159	858				
Congenital anomalies	11	10	12	16.27 (< 0.001)	0.26 (NS)	9.93 (< 0.005)	13.68 (< 0.001)
All other patients	258	187	987				
Pneumonia	42	27	93	10.19 (< 0.01)	0.33 (NS)	6.02 (< 0.02)	3.52 (< 0.10)
All other patients	227	170	906				

Comparisons are limited to those patients over 30 years of age for all diseases in this table except idiopathic cardiomyopathy, congenital anomalies, and pneumonia, where comparisons include all those over ten years of age.

*Patients with *possible* coronary artery disease are excluded.

†Patients with *possible* hypertensive cardiovascular disease (HCVD) are excluded.

Comparisons also are shown in Table 2 for the frequency of each disease among those individuals having type A and type A(−) G6PD, type A and type B G6PD, and type A(−) and type B G6PD. There is no significant difference in distribution of any disease between patients with type A and those with type A(−) G6PD except for a greater frequency of varicose veins, including hemorrhoids, for those with type A(−) G6PD at a low level of significance. With the exceptions of varicose veins and of phlebitis, distribution of the diseases shown in Table 2 differs significantly, and in the same direction, for type A G6PD patients compared with those having type B G6PD and for type A(−) G6PD patients compared with those having type B G6PD.

Coronary Artery Disease

Diagnostic criteria for myocardial infarction were restricted to a history of chest pain or acute left ventricular failure and electrocardiograms showing evolution of an infarct or residual abnormalities from an old infarct. In this report, the few patients given a diagnosis of coronary artery insufficiency by the examining physicians are grouped with those having myocardial infarction. The criterion for diagnosis of angina pectoris was anterior chest discomfort occurring with exertion and relieved by rest or nitroglycerin. Though not a required criterion for diagnosis, some type of electrocardiographic abnormality was present for all persons who had angina pectoris and type A or A(−) G6PD and for 56 of the 63 angina patients with type B G6PD.

Diagnosis of *possible* coronary artery disease was made on the following bases: chest discomfort possibly of cardiac origin but inadequately described and not attributed to coronary disease by the examining physician, well-described chest discomfort somewhat suggestive of coronary artery disease but atypical and not accompanied by electrocardiographic evidence, abnormal electrocardiograms suggesting coronary artery disease in patients with no history of chest discomfort or left ventricular failure, and congestive heart failure of undetermined cause in elderly patients.

Coronary artery disease, old or active, is found about twice as often among the patients with type B as among those with type A and A(−) G6PD (Table 2). Coronary artery disease plus possible coronary artery disease is found about one and a half times as often in the type B G6PD group as in the type A and the type A(−) group.

Patients diagnosed as coronary artery disease and those diagnosed as possible coronary artery disease, taken as a group, caused a greater proportion of clinic admissions on the day of blood sampling among those with type B G6PD (140 patients) than among those with types A and A(−) G6PD (39 patients; $\chi^2_{[1]} = 12.7209$, $P < 0.001$). When the 16 coronary artery disease patients with type A, the 11 with type A(−), and the 127 with type B G6PD are compared with the 52 healthy Austin Negro males with type A, the 25 with type A(−), and the 127 with type B G6PD, $\chi^2_{[2]} = 17.2926$, $P < 0.001$. In a comparison of patients having coronary artery disease plus

TABLE 3. CERTAIN ASPECTS OF CORONARY ARTERY DISEASE IN PERSONS WITH DIFFERENT G6PD TYPES

	Type A G6PD	Type A(−) G6PD	Type B G6PD
Total with coronary artery disease	16	11	125
Myocardial infarction	7	6	62
Angina pectoris	9	5	63
Mean age at time of this study (years)	56.6	61.0	58.8
Mean age at onset of symptoms (years)	53.5	59.3	56.1
Mean duration of coronary artery disease (years)	3.1	1.7	2.7
HCVD or possible HCVD	11 (69%)	9 (82%)	60 (48%)
No HCVD or possible HCVD	5	2	65
Diabetes mellitus	5 (31%)	1 (9%)	19 (15%)
No diabetes mellitus	11	10	106

those having possible coronary artery disease with the healthy Austin group, $\chi^2 = 14.5426$, $P < 0.001$.

About half the coronary artery disease for those with each G6PD type is myocardial infarction (Table 3). Mean age of coronary disease onset (Table 3) and mean duration of the disease do not differ markedly for those with the three G6PD types. Hypertensive disease, including both possible and definite hypertensive cardiovascular disease (HCVD), is associated more often with coronary artery disease among patients with the variant G6PD types than among those with type B G6PD (Table 3: $\chi^2_{(2)} = 6.1805$, $P < 0.05$). A test for association of diabetes mellitus with coronary artery disease shows no significant heterogeneity among those with the three G6PD types ($\chi^2_{(2)} = 3.1275$, $P \simeq 0.2$). Diabetes mellitus alone, without hypertensive disease, is associated with coronary artery disease for three patients with type A G6PD, none with type A(−) G6PD, and 11 with type B G6PD. Multiple blood pressure determinations and tests for urine glucose were available on the charts for each coronary disease patient. One or more blood glucose determinations were available for 26 of the 27 coronary disease patients with types A and A(−) G6PD and for 101 of the 125 with type B G6PD.

There is no statistically significant association between coronary artery disease and R_0 blood type, hemoglobin S, or hemoglobin C (Table 4).

Among the whole patient group over the age of 30 years, mean serum cholesterol for 38 with type A G6PD of average age 58.4 years is 207.8; for 38 with type A(−) G6PD of average age 55 years, it is 198.8; and for 165 with type B G6PD of average age 57.9 years, it is 217 mg/100 ml. On analysis of variance with $n_1 = 2$ and $n_2 = 243$, heterogeneity in these

TABLE 4. RH BLOOD TYPES AND HEMOGLOBIN TYPES RELATED TO CORONARY ARTERY DISEASE AND TO HCVD

	Rh blood types				Hemoglobin types						
	R_o	Other Rh types	χ^2	P	A	AS	χ^2	P	AC	χ^2	P
All patients with coronary disease	77	63	0.0010	NS	131	12	0.1224	NS	4	0.0123	NS
All patients with possible coronary disease	126	91			172	11			9		
All patients with no coronary disease	452	372			773	63			22		
All patients with HCVD	247	170	3.1061	0.1	366	30	0.2411	NS	19	6.3202	0.02
All patients with possible HCVD	93	87			157	11			5		
All patients with no HCVD	312	269			536	48			11		

Patients with possible coronary artery disease and patients with possible HCVD are not included in calculating values for chi square.
NS means not statistically significant.

mean values is suggested by a large variance ratio ($F = 3.54$, $P < 0.05$). Separate comparison of serum cholesterol values for patients having type A with those having type B G6PD shows no significant difference ($n_1 = 1$, $n_2 = 205$, $F = 0.772$). On comparison of serum cholesterol means for patients with type A(−) and type B G6PD, $F = 3.40$, $0.05 < P < 0.1$.

Hypertensive Cardiovascular Disease

Patients with hypertensive cardiovascular disease (HCVD) are found more often in relation to nonhypertensive patients among those with type A and those with type A(−) G6PD than among those with type B G6PD (Table 2). A similar difference is found on comparing patients having HCVD plus those having possible HCVD with nonhypertensive patients. As a cause for clinic admission on the day of blood sampling, however, patients with HCVD plus those with possible HCVD did not differ significantly among those with type A and A(−) G6PD as a group (130 patients) and those with type B G6PD (249 patients), $\chi^2 = 0.2767$, $P \simeq 0.6$. From the data in Table 2, it is apparent that 38% of the HCVD patients and 38% of the patients with possible HCVD have type A or type A(−) G6PD. This proportion is very close to the 37.8% of healthy Austin Negro males who have type A and type A(−) G6PD combined. On comparison of the numbers with each G6PD type having HCVD with the numbers of persons having each G6PD type among the Austin men, no significant difference is found ($\chi^2_{(2)} = 1.21$). For the same comparison, using numbers of patients having HCVD plus numbers having possible HCVD, $\chi^2_{(2)} = 1.20$.

For this study, HCVD has been defined as occurring in persons whose diastolic blood pressure is consistently 100 or higher, before treatment, and who have in addition at least one of the following: any degree of hypertensive vascular change in the retina, left ventricular enlargement on chest X ray, or

a left ventricular hypertrophy pattern on the electrocardiogram. Possible HCVD has been arbitrarily defined as occurring in persons whose diastolic blood pressure ranges between 90 and 100 and who may or may not have other evidence of hypertensive disease. Excluded from the HCVD and possible HCVD categories are persons with hypertension of presumably known cause: in this study, those with chronic pyelonephritis or glomerulonephritis. Screening tests for pheochromocytoma, hyperaldosteronism, and unilateral renal disease were commonly employed, but none of these conditions was found.

Histories on 74 HCVD patients with type A, 52 with type A(−), and 204 with type B G6PD are adequate for estimating the date of hypertension onset. For those with type A G6PD, mean age at estimated onset of hypertension was 55.8 years, and mean age at time of this study was 60.4 years. For those with type A(−) G6PD, the corresponding mean ages are 52.6 and 59.5 years; for those with type B G6PD, 52.8 and 58.1 years. Mean duration of known blood pressure elevation is 4.6 years for those with type A, 6.9 years for those with type A(−), and 5.3 years for those with type B G6PD.

There is no statistically significant association between HCVD and R_o blood type or hemoglobin S, but hemoglobin C is associated with HCVD with a greater than expected frequency (Table 4).

Idiopathic Cardiomyopathy

All diagnoses of idiopathic cardiomyopathy are those of the examining physicians, including the cardiology consultants who saw most patients given this diagnosis. These patients had evidence of congestive heart failure without apparent cause. Cardiomyopathy was considered by the examining physicians to be related to chronic alcoholism in three of the four patients with type B and in two of the 14 patients with types A and A(−) G6PD. Viral myocarditis was considered a possible cause of cardiomyopathy for five of the 14 cases with types A and A(−) G6PD because of nonbacterial respiratory infections associated with the onset of congestive failure. No etiology was apparent for the other cases. None had a diagnosis of subaortic valvular stenosis, although right and left heart catheterization was performed for only two with type B and 4 with types A and A(−) G6PD. The preponderance of the idiopathic cardiomyopathy cases among the patients with the G6PD variants is indicated in Table 2.

Symptoms related to idiopathic cardiomyopathy also caused clinic admission on the day of blood sampling more often for those with the G6PD variants (12 patients) than for those with type B G6PD (two patients), $\chi^2_{(1)}$ (corrected) = 12.75, $P < 0.001$. Numbers of patients with idiopathic cardiomyopathy compared with numbers of individuals having each G6PD type in the healthy Austin group also suggests an association between idiopathic cardiomyopathy and the G6PD variants, $\chi^2_{(2)}$(corrected) = 8.6899. $P = 0.02$. Of all the patients with idiopathic cardiomyopathy, six have R_o blood type and 12 have other Rh types. Two have hemoglobin AS; the other 16 have hemoglobin A.

We have examined one Caucasian with this diagnosis. She has red cell

TABLE 5. DISEASES OCCURRING WITH SIMILAR FREQUENCY IN RELATION TO RED CELL G6PD TYPES

Percentages represent occurrence of each disease among all patients with the indicated G6PD type. Probability is indicated in parentheses beneath χ^2 values. NS means not statistically significant.

	Number of Patients			Tests for heterogeneity χ^2, 2 df
	Type A G6PD	Type A(−) G6PD	Type B G6PD	
Aortic insufficiency	12 (5.1%)	15 (9.2%)	52 (6.0%)	3.07 (NS)
All other patients	223	148	818	
Bronchopulmonary disease, chronic, nontuberculous	28 (10.4%)	18 (9.1%)	92 (9.2%)	0.379 (NS)
All other patients	241	179	907	
Carcinoma	7 (2.6%)	3 (1.5%)	45 (4.5%)	5.26 (< 0.10)
All other patients	262	194	999	
Cataract, senile	10 (4.3%)	15 (9.2%)	43 (4.9%)	5.61 (< 0.10)
All other patients	225	148	827	
Cerebral thrombosis	19 (8.1%)	15 (9.2%)	82 (9.4%)	0.400 (NS)
All other patients	216	148	788	
Diabetes mellitus	34 (12.6%)	19 (9.6%)	117 (11.7%)	1.05 (NS)
All other patients	235	178	882	
Duodenal ulcer	20 (7.4%)	11 (5.6%)	47 (4.7%)	3.16 (NS)
All other patients	249	186	952	
Epilepsy	10 (3.7%)	7 (3.6%)	32 (3.2%)	0.204 (NS)
All other patients	259	190	967	
Glaucoma	7 (3.0%)	6 (3.7%)	21 (2.4%)	0.710 (NS)
All other patients	228	157	849	
Gout, primary	6 (2.6%)	3 (1.8%)	15 (1.7%)	0.687 (NS)
All other patients	229	160	855	
Infectious hepatitis	7 (2.6%)	6 (3.1%)	14 (1.4%)	4.11 (NS)
All other patients	262	191	985	
Inguinal hernia	22 (8.2%)	18 (9.1%)	56 (5.6%)	4.77 (< 0.10)
All other patients	247	179	943	
Laennec's cirrhosis	4 (1.5%)	5 (2.5%)	14 (1.4%)	1.39 (NS)
All other patients	265	192	985	

TABLE 5. (Continued)

	Number of Patients			Tests for heterogeneity χ^2, 2 df
	Type A G6PD	Type A(−) G6PD	Type B G6PD	
Malignancy, all types	9	9	52	1.63
	(3.4%)	(4.6%)	(5.2%)	(NS)
All other patients	260	188	947	
Rheumatic fever or mitral stenosis	4	3	12	0.226
	(1.5%)	(1.5%)	(1.2%)	(NS)
All other patients	265	194	987	
Schizophrenia	5	4	14	0.599
	(1.9%)	(2.0%)	(1.4%)	(NS)
All other patients	264	193	985	
Syphilis: positive VDRL	62	43	241	0.162
	(33.7%)	(32.8%)	(32.2%)	(NS)
negative VDRL	122	88	508	
Thyroid diseases	8	7	24	1.21
	(3.4%)	(4.3%)	(2.8%)	(NS)
All other patients	227	156	846	
Tuberculosis, pulmonary	2	5	28	3.88
	(0.7%)	(2.5%)	(2.8%)	(NS)
All other patients	267	192	971	

Comparisons are limited to those patients over 30 years of age for aortic insufficiency, cataract, cerebral thrombosis, glaucoma, gout, and thyroid diseases. For other diseases in this table, comparisons include all those over ten years of age.

G6PD deficiency with enzyme activity about 20% of normal and type B G6PD on electrophoresis.

Other Cardiovascular Conditions

The apparently greater frequency of thrombophlebitis, including pulmonary emboli assumed by the examining physicians to have arisen from silent phlebitis (Table 2), among those with the G6PD variants is not apparent when numbers with thrombophlebitis for the three G6PD types are compared with numbers having the three G6PD types in the Austin group, $\chi^2_{[2]}$ (corrected) = 2.4922. Heterogeneity is still indicated when those having varicose veins, including hemorrhoids, among the three G6PD types are compared with the healthy Austin groups, $\chi^2_{[2]}$ (corrected) = 8.6550, $P < 0.02$.

Aortic insufficiency, considered by the examining physicians to be of either luetic or unknown origin, is present with similar frequency among those with each G6PD type (Table 5). Four of 15 with type A and A(−) G6PD and 15 of 52 with type B G6PD with aortic insufficiency have anginal-type pain. These patients are not included as coronary disease or possible coronary disease. Acute rheumatic fever and mitral stenosis are found with similar frequency for those with each G6PD type.

In view of the low coronary artery disease frequency among those with type A and type A(−) G6PD, the similar frequency of cerebral thrombosis for all

G6PD types is noteworthy. In the group with type B G6PD, HCVD, possible HCVD, or diabetes mellitus are associated with cerebral thrombosis more often (68 of 82 patients) than with coronary artery disease (52 of 125 patients; $\chi^2_{(1)} = 13.72$, $P \simeq 0.001$).

Because of a possible vascular etiology for Paget's disease of bone (Reifenstein, 1958), the apparently greater frequency of this condition among those with type A and A(−) G6PD, seven cases, compared with those having type B G6PD, three cases, is mentioned. The small number of cases does not justify statistical analysis.

Other Diseases With Different Frequencies in Relation to G6PD Types

A varied mixture of congenital anomalies appears more often among the patients with type A and with type A(−) G6PD than among those with type B G6PD (Table 2).

This difference is also apparent when frequency of G6PD types in the healthy Austin group is used for comparison. Heterogeneity is indicated by $\chi^2_{(2)} = 10.1693$, $P < 0.01$.

The 21 congenital anomalies in the group with types A and A(−) G6PD are one Taussig-Bing syndrome; one Down's syndrome with congenital heart disease; one congenital heart disease, type not determined; one pulmonary stenosis and kyphoscoliosis; one congenital complete heart block; two cases of solitary renal cyst; one familial telangiectasia with multiple renal cysts; one case of polycystic kidneys; one congenital absence of the right kidney; one congenital nystagmus (with albinism); one bilateral external strabismus with nystagmus (with partial albinism); one congenital esotropia; one congenital clubbing of fingers (with constitutional hyperbilirubinemia); one hairlip; one Marfan's syndrome; one Klinefelter's syndrome with type A G6PD and one with type A(−)B G6PD; one polypoid hemangioma of the vocal cord; one cavernous hemangiomatosis of the liver; and one branchial cleft cyst. For the 12 persons with type B G6PD, the anomalies are one pulmonic subvalvular stenosis with hypertrophy of the outflow tract; one tetralogy of Fallot; one of multiple congenital heart defects, not further identified; one of congenital strabismus; one with type A(−)B G6PD with congenital esotropia, also listed in the type A and A(−) G6PD group and not considered Klinefelter's syndrome because of normal genitalia and several offspring; one Klinefelter's syndrome with type A(−)B G6PD, also listed in the type A and A(−) group; one congenital solitary kidney; one bifid renal pelvis; two cases of polydactyly; one with bilateral hammer toes; and one with congenital talipes varus.

A history of pneumonia is found significantly more often among those with type A G6PD than among those with type B, and a similar trend is found for those with type A(−) G6PD compared with those having type B, which does not quite reach significance at the 5% level (Table 2).

The pneumonias, however, do not differ significantly among those with the three G6PD types as a cause for clinic admission on the day of blood sampling nor does comparison of the pneumonia cases for each G6PD type with the healthy Austin group show evidence of heterogeneity ($\chi^2_{(2)} =$

1.60, $P \simeq 0.45$). Bacterial pneumonias, those of undetermined etiology usually presumed to be viral by the examining physicians, and a past history of "pneumonia" from the patient, usually without further elaboration, are included in this category.

Skin biopsy diagnoses of hyperkeratosis or of hyperkeratosis and parakeratosis had been made for five patients, four of whom have type A(−), 1 type A, and none type B G6PD.

Diseases With Similar Distribution in Relation to G6PD Types

Diseases occurring with similar frequency for those with each G6PD type are listed in Table 5. Of particular interest in view of the different coronary artery disease frequencies for males with type B G6PD compared with those having the G6PD variants is the similar distribution of diabetes mellitus among those with each red cell G6PD type. This similarity is also apparent on comparison of the number for each G6PD type having diabetes mellitus with the number in the healthy Austin group having each G6PD type ($\chi^2_{[2]} = 1.60$, $P \simeq 0.45$). Likewise, on comparison with the Austin group, no evidence is found for heterogeneity in distribution of any of the other diseases listed in Table 5 among the three G6PD types except carcinoma. For this comparison, $\chi^2_{[2]}$ (corrected) = 6.05, $P < 0.05$. Numbers of pernicious anemia, polycythemia, and Boeck's sarcoid cases were insufficient for statistical analysis.

DISCUSSION

On statistical examination of these data, the possibility appears that the considerable deficiency of patients with coronary artery disease in the type A and A(−) group might cause the apparent excess of some other diseases in this group when disease frequency is obtained by comparing the number with one disease against the number of all other patients in that G6PD group. Also the proportion of the outpatients with type A and A(−) G6PD combined, 31.8% of 1,465 outpatients, appears a little smaller than the 34.8% of 135 Oklahoma Negro males from birth to 13 years of age reported by Kirkman and Hendrickson (1963), the 33.8% of 311 randomly selected, unrelated Maryland Negro males reported by Boyer *et al.* (1962) and the 37.8% of 204 unrelated, healthy men from Austin. Differences in distribution of the G6PD types are not statistically significant when the outpatients are tested against the Oklahoma group and against the Maryland group by chi square test, but heterogeneity is suggested on comparison of the outpatients with the Austin group. Chi square of 5.86 just misses the 5% level of significance. This observation could imply that the G6PD variants are less common in the general Negro male population of Dallas and Houston from which the patients are drawn than in the population of Austin. Equally likely is the possibility that a lower frequency of some common disease, such as coronary artery disease, among the group with type A and A(−) G6PD results in a lower proportion of that group appearing as clinic patients than of the group with type B G6PD.

By all tests employed, coronary artery disease is found significantly less often among those with type A as well as type A(−) G6PD than among those

with type B G6PD. Because distribution of patients with HCVD or of patients with HCVD plus those with possible HCVD does not differ significantly when the patients having the three G6PD types are tested against the individuals in the Austin group, it seems likely that the apparent preponderance of those with HCVD or possible HCVD in the A and A(−) G6PD group is a statistical artifact caused by the deficiency of coronary disease patients in that group.

The apparent excess numbers of patients with thrombophlebitis and of those with the pneumonias also disappear when they are compared with the G6PD distribution in the Austin group. On the other hand, significantly greater numbers of patients with idiopathic cardiomyopathy, the congenital anomalies, and varicose veins persist among those with type A and A(−) G6PD on comparison with the Austin group, and the trend toward fewer patients with carcinoma among those with type A and A(−) G6PD becomes statistically significant on comparison with the Austin individuals.

This study does not rule out the possibility that coronary artery disease might be lethal earlier in its development among persons with types A and A(−) G6PD for unknown reasons. This could result in a greater loss of patients with the G6PD variants from coronary disease deaths before they reach the outpatient clinics. Against this possibility is the similar duration of coronary disease, 2.7 years for type B men and 2.5 years for type A and A(−) men combined.

The even distribution of cerebral thrombosis cases among those with the three G6PD types is not consistent with the differences in coronary disease distribution for those with the different enzyme types. Negro populations (Phillips and Burch, 1960; Reef and Isaacson, 1962) and Sephardic Jewish populations (Kallner, 1962) which have low coronary disease frequencies do not have proportionately reduced frequencies of cerebrovascular disease, however.

The possibility of some direct relationship between G6PD function and coronary artery disease would be enhanced if those with G6PD variants in populations such as Israel, Greece, or Sardinia should be found to have less coronary artery disease than those with the usual form of the enzyme. In this regard, a study of G6PD types in animal strains with high and low susceptibility to atherosclerosis, such as White Carneau and Show Racer pigeons (Lofland and Clarkson, 1965) might also be worthwhile. Red cell G6PD activity varies considerably among animal species and sometimes among strains of the same species (Salvidio *et al.*, 1963).

G6PD deficiency would presumably account in some manner for any direct relationship between G6PD types and a given disease. Enzyme activity for red cells with type A(−) G6PD is deficient, being 8–20% of the activity for red cells with type B G6PD (Kirkman, McCurdy, and Naiman, 1964). Red cell enzyme activity for type A G6PD has been reported as normal (Boyer *et al.*, 1962; Kirkman and Hendrickson, 1963), although we have found mean G6PD activity for red cells from 58 Negro males with type A to be about 80%

of mean activity for red cells from 165 with type B G6PD (unpublished data). The difference is significant by analysis of variance ($P < 0.001$).

Since type A, which is only slightly deficient in the red cell, parallels type A(−) G6PD in disease associations, and, since types A and A(−) G6PD have been reported only among Negroes, the possibility must be considered that a group with types A and A(−) G6PD may have a larger portion of Negro genes relative to Caucasian genes than a group with type B G6PD. In this situation, the direct association of a disease might be with the product or products of these unknown genes rather than with type A or A(−) G6PD. Against the possibility of such gene stratification is the even distribution of other genetically determined features found mainly in Negroes, R_o blood type and the S and C hemoglobin variants, among the patients with the different G6PD types (Table 1). Similarly, the even distribution of R_o blood type and the hemoglobin variants among those with and those without coronary artery disease (Table 4) increases the likelihood that the lower frequency of coronary disease among the patients with the G6PD variants may be directly related to the variants.

Since phlebitis and pulmonary embolism occur as often among patients with type A and with type A(−) G6PD as among those with type B, speculation on a mechanism for any direct relation between G6PD types and coronary artery disease should probably focus on areas other than blood coagulation. The same G6PD type found in the red cell is present in other tissues which have been studied (Davidson *et al.*, 1963; Linder and Gartler, 1965), and the conversion rate of intravenous glucose with carbon-1 and also with carbon-6 labels to labeled CO_2 is diminished in persons with type A(−) G6PD to a degree that implies expression of G6PD deficiency in tissues in addition to the hematopoietic system (Carson *et al.*, 1964).

Pentose phosphate pathway function has been demonstrated in the isolated pigeon aorta, where a large proportion of the total glucose metabolism was found to occur via this pathway (Lofland and Clarkson, 1965). G6PD activity has been found in samples of human aorta, coronary artery, and pulmonary artery by Kirk (1964), who reports that activity of this enzyme from aortas of women is significantly lower than that from aortas of men. This sex difference is similar for a smaller number of coronary artery samples.

Coronary atherosclerosis could possibly be influenced by altered lipid metabolism as a result of the TPNH deficiency which is secondary to G6PD deficiency, at least in the red cell (Schrier *et al.*, 1958). TPNH is a coenzyme for several reactions involved in the synthesis of fatty acids and cholesterol (Siperstein, 1959). G6PD-deficient red cells have diminished lipid content presumably because of their diminished ability to generate TPNH from TPN (Tarlov *et al.*, 1962). A similar situation might exist in arterial endothelium of G6PD-deficient persons. Arterial tissue is known to synthesize cholesterol (Siperstein *et al.*, 1951), although the relationship of this locally synthesized cholesterol to atherosclerosis remains uncertain (Moses, 1963). If diminished lipid synthesis were a widespread phenomenon involving many tissues of per-

sons with G6PD variants, one manifestation might be a low serum cholesterol content. This is the trend, at not quite a 5% level of significance, for the A(−) men in this study on whom serum cholesterol had been determined. This trend is difficult to evaluate because those with type A(−) G6PD on whom serum cholesterol determinations were available had a mean age of three years less than those with type B G6PD on whom serum cholesterol had been determined. Tarlov *et al.* (1962) found the reverse in a study of healthy Negro males whose mean serum cholesterol was higher for those with G6PD deficiency than for those with normal G6PD activity.

This series does not include an adequate number of persons with idiopathic cardiomyopathy to provide interpretations for the finding of a greater number with this diagnosis among patients with the variant forms of G6PD. The presence of G6PD deficiency in the one Caucasian examined with idiopathic cardiomyopathy, the higher incidence of some types of primary cardiomyopathy among Negro people (Phillips and Burch, 1960; Parry, 1965), and reports that chemical agents including the 8-aminoquinoline compound pamaquine (Adams *et al.*, 1962), 5-hydroxytryptamine (Ojo and Parratt, 1966), and ingestion of certain plants (Dewan *et al.*, 1965; Brink *et al.*, 1965) can produce myocardial damage in various species including man provide further suggestive evidence for a relation between cardiomyopathy and the G6PD variants. The very slight activity of G6PD in the normal myocardium (Dawson and Romanul, 1964) suggests that any relation of G6PD variants to cardiomyopathy might be extra-myocardial, possibly via altered metabolism of various chemical agents due to TPNH deficiency secondary to G6PD deficiency. TPNH is involved in a number of reactions concerned with drug metabolism (Brodie, 1956).

No conclusions can be drawn from the greater proportion of various congenital anomalies among those with the G6PD variants than among those with type B G6PD. This mixture of conditions is difficult to evaluate statistically. Except for polydactyly and congenital inguinal and umbilical hernias, the trend among Negro populations is for a lower frequency of congenital anomalies than among Caucasians (Shapiro *et al.*, 1958; Gittelson and Milham, 1965). Possible involvement of G6PD in structural development, at least of *Neurospora,* is suggested by the report of Brody and Tatum (1965) that a deficient G6PD variant of *Neurospora crassa* seems to determine the growth of that organism in colonial as opposed to the usual filamentous form of the wild type.

The slight excess of carcinoma cases in the type B G6PD group suggested on comparing the outpatients with the healthy Austin group is currently being investigated by study of patients from several hospitals.

Frequencies of AS, S, and AC hemoglobin types in this study are comparable to those reported by others for American Negroes (reviewed by Wintrobe, 1961). The finding of R_o blood type in 55.2% of these outpatients is similar to that of 52.9% recently found in a Georgia Negro population (Cooper *et al.*, 1963).

Type B(−) G6PD, found in four individuals, has previously been reported rarely among Negroes (Boyer *et al.*, 1962; Carson and Frischer, 1966; Pinto *et al.*, 1966). Enzyme activity for the four with type B (−) G6PD is within the range of those having type A(−) G6PD. Kinetic and lability studies, to be reported elsewhere, are normal for two of the B(−) individuals, characteristic of the Mediterranean G6PD variant for one, and are not available for the fourth.

SUMMARY

Coronary artery disease has been found less frequently among Negro male outpatients with type A or type A(−) red cell glucose-6-phosphate dehydrogenase (G6PD) variants than among those with type B G6PD of comparable age. Diabetes mellitus is evenly distributed among patients with the different G6PD types. The apparently greater frequency of hypertensive cardiovascular disease among those with the G6PD variants is probably a statistical artifact caused by the decreased frequency of coronary artery disease patients in the G6PD variant group.

Interpretation of the negative association between coronary artery disease and the red cell G6PD variants includes the possibility of altered lipid metabolism, either locally in the arterial wall or generally in many tissues for persons having the G6PD variants.

Higher frequencies of idiopathic cardiomyopathies and of miscellaneous congenital anomalies have been found among patients with the variant G6PD types than among those with type B G6PD.

ACKNOWLEDGMENT

The authors wish to thank Mr. John Brooks and Miss Thelma Irvin for collection of hospital samples; Mrs. Eva Marie Mosby for obtaining samples on the healthy Austin group; Mrs. Ann Geisler and Mrs. Patricia Tannler for technical assistance; Mrs. Florence Hall and Mrs. Maggie Newsom for obtaining the many hospital records for review; and Drs. Harold Brown, Harold Dobson, and Milton Hejtmancik for permission to study their clinic patients.

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