Gene Frequencies and Microdifferentiation among the Makiritare Indians. III. Nine Erythrocyte Enzyme Systems

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The two previous papers in this series have described the blood groups (Gershowitz et al. 1970) and serum protein types (Arends, Weitkamp et al. 1970) of the Makiritare Indians of Venezuela and Brazil, based on seven collection sites. In the present paper we will describe the findings with respect to nine erythrocytic enzymes. The geographical relationships of the localities to one another, as well as certain descriptive material concerning each locality, are to be found in Gershowitz et al. (1970).

METHODS

The circumstances of the collection and handling of the blood samples prior to arrival in the laboratory are detailed in Gershowitz et al. (1970). Upon receipt, the samples collected in 1967 (Juduwaduña village) were stored at 4° C for not more than three weeks before typing was begun. However, because the samples from the remaining villages were part of a much larger collection received in a period of less than two months, it was not possible in some instances to begin typing until three months after venipuncture. The red cells which could not be immediately typed were therefore washed three times in isotonic saline, frozen in 1-cm^3 aliquots, and stored at −70° C. Such storage proved perfectly adequate for all of the enzymes examined (see below). Hemolysates were prepared from washed cells by shaking for 15 minutes with 1.5 volumes of distilled water and 1.5 volumes of toluene, the toluene layer being discarded. The specimens were then centrifuged at 27,000 *g* for 20 minutes to remove the remaining stroma.

Samples from all of the villages were typed with respect to five red cell enzymes: acid phosphatase (AP), phosphoglucomutase (PGM), 6-phosphogluconate dehydrogenase (6PGD), adenylate kinase (AK), and an "oxidase" (see Brewer 1967). The specimens from Juduwaduña were typed for glucose-6-phosphate dehydrogenase (G6PD). The remaining villages (later collections) were not typed for G6PD, but were either typed for lactic and malic dehydrogenase (LDH, MDH) or for adenosine deaminase (ADA). The typing of the specimens from Juduwaduña was generally performed according to procedures originally described for the individual enzyme systems; that is, AP was typed by the method of Hopkinson et al. (1964), PGM by the method of Spencer et al. (1964), and AK by the method of Fildes and Harris (1966); G6PD and 6PGD were typed using the method of Shows et al. (1964) (for the typing of 6PGD, 6-phosphogluconate was substituted for glucose-6-phosphate in the staining solution). In order to proceed efficiently, the samples from the remainder of the villages were typed in a single electrophoretic system for all of the enyzmes (AP, PGM, AK, ADA, LDH, MDH, 6PGD, and "oxidase"). The details of the electrophoretic conditions and incubation media are given in Weitkamp et al. (1969).

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RESULTS

The results of classification with respect to nine red cell enzymes (representing at least 11 genetic loci) are listed in table 1. One of the enzymes (G6PD) was examined only in members of Juduwaduña village. Almost all members of the other villages were typed for AK and either ADA or LDH and MDH. There was no variability at any of the six genetic loci represented by these five enzymes. All of the specimens were typed for AP, PGM, 6PGD, and "oxidase." The gene frequencies for the variable systems (AP, PGM_1, and 6PGD) are shown in table 2. The PGM_2 and "oxidase" loci were invariable.

TABLE 1

SUMMARY OF THE ERYTHROCYTE ENZYME PHENOTYPE FREQUENCIES IN SEVEN MAKIRITARE VILLAGES

	A Judu-waduña	BD Santa Maria	C Wasaña	E Chajo-raña	F Shara-maña	G Belen	H Acanaña	Total
Acid phosphatase:								
BA	7	14	11	8	0	8	8	56
B	64	138	32	67	46	64	66	477
Not typable	0	2	0	0	0	0	0	2
Total	71	154	43	75	46	72	74	535
Phosphoglucomutase$_1$:								
1-1	67	120	30	54	38	32	43	384
2-1	2	31	13	21	8	39	28	142
2-2	2	3	0	0	0	1	3	9
Total	71	154	43	75	46	72	74	535
Phosphoglucomutase$_2$:								
1-1	71	154	43	75	46	72	74	535
Adenylate kinase:								
1-1	15	153	43	74	46	72	74	477
Lactic dehydrogenase A and B:								
Normal	8	154	43	75	46	.....	.....	326
Malic dehydrogenase	0	151	43	75	39	.....	.....	308
Glucose-6-phosphate dehydro-genase:								
B	70	.....	.....	.....	.....	.....	.....	70
Not typable	1	.....	.....	.....	.....	.....	.....	1
Total	71	.....	.....	.....	.....	.....	.....	71
6-phosphogluconate dehydro-genase:								
A	71	144	40	75	46	72	74	522
AB	0	5	0	0	0	0	0	5
A-Makiritare (50% activity)	0	2	3	0	0	0	0	5
Total	71	151	43	75	46	72	74	532
"Oxidase":								
Normal	71	154	43	75	46	72	74	535
Adenosine deaminase:								
1-1	.....	.....	.....	.....	.....	72	74	146

The frequency of the acid phosphatase *A* allele ranged from 0 to .13, the tribal average of .05 being a higher figure than was reported for 10 Venezuelan Yanomama villages (Arends et al. 1967) but lower than that reported for American Indians as far south as Mexico, as well as for Caucasians, Negroes, Orientals, and Eskimos (see Lisker and Giblett 1967). The frequency of the PGM_1^2 allele ranged from .04 to .29, the mean of .15 representing a frequency similar to that reported for other American Indians and for Caucasians and Negroes (see above references). Although adenylate kinase has not been extensively studied in the American Indian, previous reports indicate that a small number of Yanomama Indians (Arends et al. 1967) and 150 Lacondon Mayans (Bowman et al. 1967) also had only the AK 1-1 phenotype. This contrasts to a 5%–10% frequency of the AK 2-1 phenotype among Caucasians (Rapley et al. 1967; Bowman et al. 1967). The 146 Makiritare Indians examined were all

TABLE 2

SUMMARY OF THE ERYTHROCYTE ENZYME GENE FREQUENCIES IN MAKIRITARE VILLAGES

	A Judu-waduña	BD Santa Maria	C Wasaña	E Chajo-raña	F Shara-maña	G Belen	H Acanaña	Total
Acid phosphatase:								
P^A	.05	.05	.13	.05	.00	.06	.05	.05
P^B	.95	.95	.87	.95	1.00	.94	.95	.95
Phosphoglucomutase$_1$:								
PGM^1	.96	.88	.85	.86	.91	.71	.77	.85
PGM^2	.04	.12	.15	.14	.09	.29	.23	.15
6-phosphogluconate dehydrogenase:								
Pd^A	1.00	.98	.97	1.00	1.00	1.00	1.00	.99
Pd^B	.00	.02	.00	.00	.00	.00	.00	.005
Pd^{Mak}	.00	.01	.03	.00	.00	.00	.00	.005

homozygous for the ADA^1 allele of the adenosine deaminase system. Gene frequencies for the adenosine deaminase polymorphism have not been previously reported for the American Indian, but among the populations which have been surveyed, the ADA^2 allele has had a frequency of 3%–11% (Spencer et al. 1968). LDH and MDH have not had a polymorphic frequency of variants in any population studied.

The five 6PGD AB heterozygotes of Santa Maria village (BD) were all members of the same family. One 6PGD AB heterozygote has been previously reported in a Yanomama Indian (Arends et al. 1967) and four 6PGD B homozygotes among 88 Lacondon Mayans (Bowman et al. 1966). Although the variant phenotype in the family from Santa Maria is electrophoretically indistinguishable from the AB phenotype in Caucasians, it is not certain whether this represents the occurrence of a rare mutation or is another example of the same allele which has been found in polymorphic frequency in other populations. Similarly, the five individuals with decreased activity of 6PGD (as estimated from the intensity of staining on the starch gel) may erpresent examples of a phenotype found with a frequency of 0.7% in Caucasians and 0.3% in Negroes (Dern et al. 1966), or may represent a mutant unique to

the Makiritare. The two individuals from Santa Maria with the decrease in activity were a mother and son; those from Wasaña were a mother with four apparently normal children and a father and son. An exception to autosomal codominant inheritance appears to be that both parents of the mother from Santa Maria have normal 6PGD activity on examination of the starch gel. The inheritance of alleles at the other loci tested in this series of papers, however, was consistent with the parentage. Since Dern et al. (1966) point out the importance of quantitative assay to determine this phenotype, it may well be that one of the parents, as well as perhaps other individuals among the Makiritare, were mistyped in the starch gel screening procedure.

The number of "private" genetic traits encountered in the serum proteins and erythrocyte enzymes is noteworthy. In population studies of this type (involving family material) we use the term "private" for genes present with a frequency less than 1%. In practice, such a trait will be present in only one or two kindreds. Since the antisera used for blood grouping respond to known variants, the results of blood groupings are of no value in determining the frequency of rare variants. On the other hand, electrophoretic procedures may recognize any variant accompanied by a change in molecular charge. In this study, 14 systems representing at least 15 loci (equating LDH to two) were screened for electrophoretic variants, for a total of 6,930 phenotypes successfully typed. There were 13 *possible* instances of private factors, namely, three similar Gc types, five 6PGD of one type, and five 6PGD of another type. However, it should be noted that both of the 6PGD variants are presently indistinguishable from a variant found with 0.5% and 3%–5% frequency in other populations. Moreover, there were 13 similar albumin variants, a frequency just in excess of the definition "private." However, a similar and perhaps identical albumin variant has been found in the Warao Indians (Arends, Gallango et al. 1970). It is clear that the identification of a variant as truly private will at times be difficult.

Wright (1966) has discussed the manner in which such variants can be employed to obtain estimates of mutation rates. Data of this type permit obtaining indirect estimates of human mutation rates under circumstances where direct estimates are not possible. One of our long-range objectives is to accumulate sufficient data for a meaningful comparison of mutation rates in primitive and civilized man by this approach. However, before that comparison is possible, we must understand better the population structure of both primitive and civilized man, and how the differences between them, and the differences of both from the structure assumed by Wright (1966), will modify the calculations.

SUMMARY

Phenotype and gene frequencies are presented for acid phosphatase, phosphoglucomutase and 6-phosphogluconate dehydrogenase in a group of 535 Makiritare Indians from seven villages in southern Venezuela. Many of these individuals were also typed for glucose-6-phosphate dehydrogenase, adenylate kinase, lactic and malic dehydrogenases, adenosine deaminase, and "oxidase." No genetic variability was detected in the latter systems. The 6-phosphogluconate dehydrogenase system was also constant in its expression, except for two "private" variants. The group is also notable in having a low frequency (.05) of the acid phosphatase *A* allele, the value in indi-

vidual villages ranging from .00 to .13. The village frequency of the PGM^2 allele varied from .04 to .29, with a mean of .15.

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