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Genetic Studies of the Macushi and Wapishana Indians

I. Rare Genetic Variants and a "Private Polymorphism" of Esterase A

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Summary. Blood samples from 509 Macushi and 623 Wapishana Amerindians of Northern Brazil and Southern Guyana have been analyzed with reference to the occurrence of rare variants and genetic polymorphisms of the following 25 systems: (i) *Erythrocyte enzymes:* acid phosphatase-1, adenosine deaminase, adenylate kinase-k, carbonic anhydrase-1, carbonic anhydrase-2, esterase $A_{1,2,3}$, esterase D, galactose-1-phosphate uridyltransferase, isocitrate dehydrogenase, lactate dehydrogenase, malate dehydrogenase, nucleoside phosphorylase, peptidase A, peptidase B, phosphoglucomutase 1, phosphoglucomutase 2, phosphogluconate dehydrogenase, phosphohexoseisomerase, triosephosphate isomerase and (ii) *Serum proteins:* albumin, ceruloplasmin, haptoglobin, hemoglobin A, hemoglobin A_2 and transferrin. Fifteen different rare variants were detected, involving 11 of these systems. In addition, a previously undescribed variant of $ESA_{1,2,3}$ which achieves polymorphic proportions in both these tribes is described. Excluding this variant, the frequency of rare variants is 1.1/1000 in 12 510 determinations in the Macushi and 4.7/1000 in 15 396 determinations in the Wapishana. The $ESA_{1,2,3}$ polymorphism was not observed in 382 Makiritare, 232 Yanomama, 146 Piaroa, 404 Cayapo, 190 Kraho and 112 Moro. Irregularities in the intratribal distribution of this polymorphism in the Macushi and Wapishana render a decision as to the tribe of origin impossible at present. Gene frequencies are also given for previously described polymorphisms of 5 systems: haptoglobin, phosphoglucomutase 1, erythrocyte acid phosphatase, esterase D, and galactose-1-phosphate-uridyl-transferase.

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The Macushi and Wapishana Indians are currently situated in the Roraima Territory of Northern Brazil and in Southern Guyana. Although by now both tribes are quite acculturated, Layrisse et al. (1963) did not detect any non-0 or Kell positive persons in 119 Macushi from Guyana and Matson et al. (1968) in typings of 261 Macushi from Brazil observed only 2 non-0 individuals and no Kell positives. Similarly, Layrisse and Wilbert (1966) in typings of 119 Wapishana from Guyana did not observe any non-0 or Kell-positive individuals. Accordingly, the tribes were selected for inclusion in our ongoing study of the kinds and numerical representation of rare genetic variants of 25 proteins of the blood serum and erythrocyte, a rare variant being arbitrarily defined as one occurring in less than 2% of the population. Such variants are of particular interest because of their usefulness in generating both direct and indirect estimates of the rates of mutation of the cistrons encoding for these proteins, as well as in seeking evidences of the action of selection. An additional point of interest with reference to the study of these two tribes is that they are located immediately to the east of the Yanomama, in whom we have recently demonstrated an apparently "private" polymorphism of serum albumin (Tanis et al., 1974). Data from these tribes were therefore particularly important to understanding the dynamics of this polymorphism.

Preliminary field work was carried out in 1972 and followed by more extensive studies in 1974. In addition to the examination of the specimens for rare variants of 25 proteins, typings have been performed with respect to the occurrence of genetic polymorphisms of 12 additional systems, to extend the earlier observations of Layrisse and Wilbert (1966) and Matson et al. (1968) and so improve the potential usefulness of these tribes in efforts to understand the genetic interrelations of the Amerindians of South America. In this paper we present only the results of the typings for variants of the following 25 systems (locus symbol in parentheses):

i. Erythrocyte Enzymes. Acid phosphatase-1 (ACP_1), adenosine deaminase (ADA), adenylate kinase-k (AK_1), carbonic anhydrase-I (CA_1), carbonic anhydrase-II (CA_{II}), esterases A_1, A_2, and A_3 ($ESA_{1,2,3}$), esterase D (ESD), galactose-1-phosphate uridyltransferase ($GALT$), isocitrate dehydrogenase (ICD_S), lactate dehydrogenase (LDH_A and LDH_B), malate dehydrogenase (MDH_S), nucleoside phosphorylase (NP), peptidase A ($PEPA$), peptidase B ($PEPB$), phosphoglucomutase 1 (PGM_1), phosphoglucomutase 2 (PGM_2), phosphogluconate dehydrogenase (PGD), phosphohexoseisomerase (PHI), and triosephosphate isomerase (TPI).

ii. Serum Proteins. Albumin (ALB), ceruloplasmin ($CRPL$), haptoglobin (HP), hemoglobin A ($HGB\alpha$ and $HGB\beta$), hemoglobin A_2 ($HGB\alpha$ and $HGB\delta$), and transferrin (TF).

The products associated with at least 27 genetic loci are represented. For five of these systems known genetic polymorphisms were encountered, and these data will also be presented. A preliminary and partial report on the findings regarding rare variants in the material collected in 1972, which involved samples from 2 Macushi villages (26AB, 26CD) and 1 Wapishana village (27A), has been

included in a previous paper (Tanis et al., 1973); these findings will be repeated and extended in this paper in the interests of a comprehensive treatment.

Unusual aspects of the present observations are the discovery of a "private polymorphism" of Esterase A in these two tribes, and the demonstration of an unusually high frequency of rare variants in one village. In the companion paper to this communication, we will present the findings with respect to the 12 additional polymorphic systems which were studied, attempting to use these observations to understand the pattern of gene flow between the two tribes as well as the possibility of the introduction of some of the rare variants by non-Indians.

The Tribes

The Macushi. Carib-speaking tribes extend in a broad band from French Guyana west to the Amazonas Territory of Venezuela. The Macushi belong to the geographical grouping referred to as the Central Caribs by Farabee (1924). Currently they occupy an area roughly bounded by latitudes 3° to 5° N and longitudes 59° 40′ to 61° 20′ W. As shown in Figure 1, their tribal boundaries overlap substantially with those of the Wapishana. The earliest reports, 200 years ago, place them on the Upper Essequibo River of Guyana, from which region they have been displaced northwesterly by the Wapishana (Gillin, 1948). Whether this was a total displacement, or whether the southeastern Macushi simply withdrew into territory already occupied by other Macushi, is unclear. Schomburgk (1847—1848) and Brett (1968) speculate that the Macushi may have been the same Carib tribe encountered by Raleigh in 1595 on the lower Orinoco, implying a considerable southernly migration some 300 years ago.

This is savannah country, drained by the Rio Branco and its tributaries, with the Sierra Pacaraima to the north. The Portugese penetrated into this relatively pleasant area at the end of the eighteenth century, and it became known as good cattle country. The headwaters of the Rupununi River, a tributary of the Essequibo, the principal river of Guyana, are a day's journey by trail from the Tacuto, a northeastern tributary of the Rio Branco, this situation providing an "inland waterway-trailway" from the Atlantic Coast of Guyana to the Rio Negro. In colonial times there was a garrison on the Tacutu, to protect Brazil against incursions from the north. The Macushi have thus been in contact with Caucasians of various nationalities and Negroes (the latter originally introduced as slaves) for over 200 years.

One of us (E.C.M.) estimates that there are about 3500 Macushi in Brazil and 500 in Guyana, distributed for the most part in 68 villages in Brazil and 8 in Guyana. Until recently a larger number of villages were in Guyana, but a sharp disagreement with the government in 1968 caused many of the Guyanese Macushi to move into Brazil—the latest of the many events tending to obscure their tribal structure. Village size ranges from 40 to 300 or (temporarily) 400 persons. Two dialects are recognizable, one spoken in the region of the Maú River and eastward into Guyana, the other spoken to the west. Traditionally their kinship system was of the bifurcate merging type and the terminology for cousins

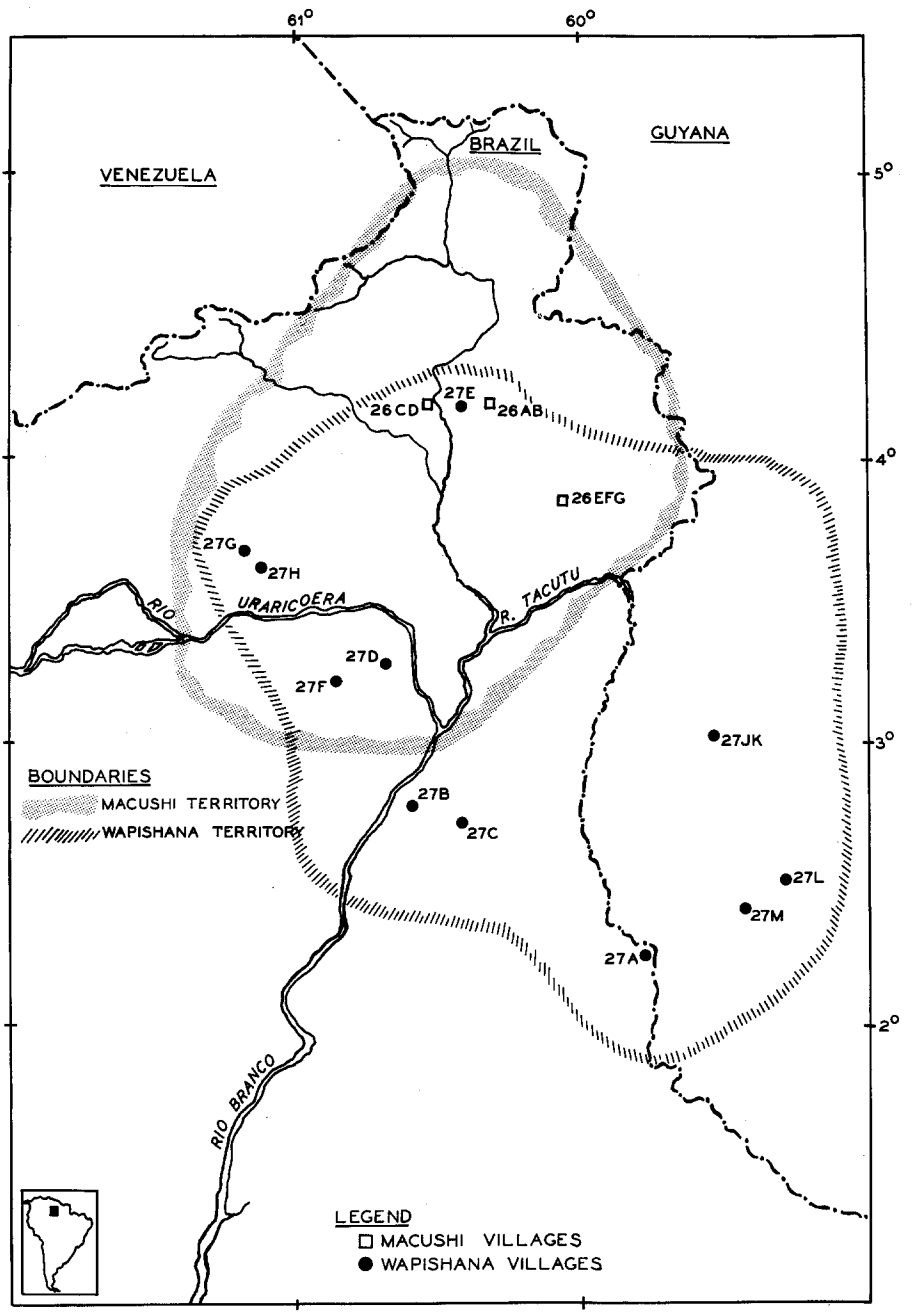

Fig. 1. Tribal distributions of the Macushi and Wapishana and locations of villages studied

of the Iroquois type. Formerly sororal polygyny was common. The degree of acculturation is variable, some of the villages in the northern part of their distribution still remaining quite isolated, whereas in their southern distribution many Macushi work for ranchers, often living on the ranch or in the scattered towns of the area. Most of them, however, are subsistence farmers.

Since the time of Schomburgk (1847—1848) the explorers of this region have commented on admixture between the Macushi and the Wapishana to their southeast (Coudreau, 1887 and Ule, 1913, quoted from Layrisse and Wilbert, 1966; Koch-Grünberg, 1917). In addition, admixture of the Macushi with their northern neighbors, the Taurepan (a subdivision of the Pemon) and the Ingariko, both Carib-speakers, also occurs (Thomas, 1973; Migliazza, unpublished).

The Wapishana. This Arawak speaking tribe currently occupies a region approximately bounded by latitudes 2° and 4° N and longitudes 59° and 61° (see Fig. 1). Gillin (1948) identified their first known concentration as the basin of the abovementioned Tacutu River. At the time of these early contacts there were at least 4 Arawak tribes in this region (Wapishana, Atorai, Amariba, Maopityan), all speaking mutually intelligible dialects, a linguistic island within an area of Carib-speaking tribes. Migliazza (1972) argues for a pre-Columbian eastward movement of Arawak speakers from the Rio Negro region, suggesting that the location ascribed to the Wapishana in the early nineteenth century was not relatively recent and predated the influx of Macushi into the same area.

The Wapishana were first contacted about the same time as the Macushi. Farabee in 1918 noted that in addition to the displacement of the Macushi mentioned above, the Wapishana were in the process of absorbing the Atorai, an eastenly Arawak tribe (there are still a few Atorai-speakers in Guyana), and Rivet (1924) mentioned they have incorporated remnants of the Paraviyana, a Carib-speaking tribe to the southwest. Farabee also mentioned several other tribes they claimed to have absorbed recently when he visited them in 1913. Their history of contacts with Caucasians are very similar to those of the Macushi.

Although at the time of the first contact with Europeans the relations of the Wapishana with the Macushi seem to have been generally hostile, and were so until the first quarter of this century, Schomburgk (1847—1848) in his justly famous expedition through this area records observing mixed villages at that time. On the basis of data supplied by Wapishana informants, marriages between members of the two tribes have occurred with increasing frequency in the past quarter century. Layrisse and Wilbert (1966) also suggest considerable Wapishana admixture with the Taurepan, a subdivision of the Pemon, presently living to the north of the Macushi. Thus, the historical evidence suggests that the Wapishana are highly admixed with other Indian tribes, and, in addition, have some 200 years of contact with Caucasians and Negroes. We shall return to the biological evidence of the results of these contacts later.

E.C.M. estimates there are about 2000 Wapishana, 1200 in Brazil and 800 in Guyana, mainly distributed among 14 villages in Brazil and 10 in Guyana. Village size ranges from 50 to 150. In general the acculturation of the Wapishana resembles that of the southern Macushi, with some families living on ranches or in towns. Their kinship system was also formerly of the bifurcate merging type and cousin terminology of the Iroquois type.

Table 1. Known Indian and non-Indian admixture in the Macushi (Ma) and Wapishana (Wa) Indians

	Ma	Wa	Ma/ Wa	NB	Ma/ NB	Wa/ NB	Ma/Wa/ NB	Taur.	Ma/ Taur.	Wa/ Taur.	Ma/Wa/ Taur.	Wa/ Atr.	Un- certain	Ad- mixture total	Total
Macushi (26)															
AB	90	0	0							0				0	90
CD	163	7	23							4				34	197
EFG	205	2	15							—				17	222
Total	458	9	38							4				51	509
Wapishana (27)															
A	2	45	1	—	—	9	—	—	—	—	—	—	5	17	62
B	4	41	3	—	1	8	3	1	2	1	—	—	—	23	64
C	4	9	1	1	4	—	—	—	—	—	—	1	—	11	20
D	2	19	4	1	1	3	8	—	—	3	—	—	—	22	41
E	7	39	21	1	—	2	—	—	—	—	—	—	—	31	70
F	1	27	2	—	—	2	—	—	—	—	—	—	—	5	32
G	0	36	12	7	—	3	—	—	—	—	—	—	—	22	58
H	2	6	2	2	—	5	—	7	2	8	8	—	—	36	42
JK	1	82	—	—	—	—	—	—	—	—	—	—	2	3	85
L	0	96												0	96
M	2	42	1	1	—	—	—	—	—	—	—	—	7	11	53
Total	25	442	47	13	6	32	11	8	4	12	8	1	14	181	623

Key: NB, Neo-Brazilian; Taur., Taurepan; Atr., Atroari

Our Sample. Blood samples have been collected from a total of 11 Wapishana and 3 Macushi villages; these figures include the pilot collections mentioned above. These villages will be designated by an alphanumeric code, the first two numbers for the tribe and the subsequent letter(s) for the village. The locations of the villages are shown in Figure 1. Samples were obtained from all available members of each village studied. The Wapishana "villages" we contacted were small and rather diffuse; we estimate that 70—80% of the inhabitants over age 6 were sampled in these villages. The Macushi villages were larger; again the sample would include 70—80% of all those in the village over age 6. Villages 26CD and 26EFG correspond to the Contão and Raposa sampled by Matson et al. (1968), who also included in their sample a mission not visited by us. Layrisse and Wilbert collected their samples of both Macushi and Wapishana in the vicinity of Lethem, a Guyanese town just east of the Brazilian border (3° 25′ N, 60° 50′ W); their sample should not overlap with ours.

The adults who were sampled were routinely queried concerning their ancestry. Individuals have been classified by their village of residence. The results, shown in Table 1, of course provide a minimal estimate of current admixture and do not speak at all to remote admixture. The term "neo-Brazilian" is customarily applied to a person resulting from a complex Caucasian/Negro/Indian admixture of varying proportions but who, with respect to the Indian component, no longer identifies himself as Indian. Note the greater incorporation of Macushi into nominally Wapishana villages than the reverse. We conclude from this only that the admixture mentioned by the early anthropologists continues to the present time. In the tabulations that follow, all persons living in Wapishana or Macushi villages have been scored as Indians, i.e., no effort has been made to exclude neo-Brazilians or their children from the tabulations.

Methodology

Samples were collected in Becton-Dickinson vacutainers either without anticoagulant or containing ACD solution, and chilled as soon as possible. They were then shipped on ice to Ann Arbor, the usual elapsed time between collection and receipt being 5—7 days. In Ann Arbor they were stored in 1 cc. aliquots of cells or serum at —80° C or in liquid N_2 until typing.

The conditions employed in this laboratory for the detection of electrophoretic variants in the systems enumerated above have, with five exceptions, either been described or referenced in our previous publications (Tanis et al., 1973; Neel et al., 1976). These exceptions are CA_I and CA_{II}, $ESA_{1,2,3}$ and ESD, and GALT. Electrophoresis of CA_I and CA_{II} followed the method of Tashian (1969), but the staining technique, employing 4-methyl-umbelliferyl-acetate and fluorescein diacetate, was as described by Hopkinson et al. (1974). With respect to $ESA_{1,2,3}$ and ESD, originally both systems were visualized on the same starch gel as CA_I and CA_{II}, employing the electrophoretic conditions of Tashian (1969) but the staining conditions of Hopkinson et al. (1974). When variation was observed in the $ESA_{1,2,3}$ bands, it quickly became apparent that a satisfactory understanding of the variation could not be obtained in this fashion because of poor resolution of the bands. Accordingly, an improved routine gel system was developed to ascertain the data reported here. Electrostarch gels (Electrostarch Co., Madison, Wisc.) were run vertically at 4° C for 18 to 20 h. The best patterns were obtained using the following buffers: gel buffer containing 0.0124M Tris, 0.0033M citric acid, 0.0036M boric acid and 0.00033M lithium hydroxide; bridge buffer containing 0.44M boric acid, 0.04M Tris and 0.04M lithium hydroxide. Both buffers were adjusted to pH 7.2 with HCl. Optimum separation

occurred with 4 volts/cm as measured directly across the starch gel. $ESA_{1,2,3}$ and ESD were typed from slices of the same gel. These electrophoretic conditions resulted in an $ESA_{1,2,3}$ pattern very similar to that previously reported by Tashian and Shaw (1962).

We feel the advantage of our electrophoretic conditions is that very clear typings of both ESD and $ESA_{1,2,3}$ (Hopkinson et al., 1973) can be obtained from the same gel since at pH 7.2 the hemoglobin does not migrate into the anodal portion of the gel. The routine staining procedure for $ESA_{1,2,3}$ was that developed by Tashian (1969). However, since the completion of this work the coupling azo dye, Blue RR Salt, has been withdrawn from the market. We have found the Fast Blue RR Salt (Sigma Chemical Co.) to be a satisfactory substitute dye. The GALT gel system employed in these studies was based on the procedures reported by Sun et al. (1974) except that electrophoresis was carried out for 18 h at 3.2 volts/cm. Also, we employed Sephaphore III (Gelman Instrument Co.) cellulose acetate strips to improve the staining technique. The strips were saturated with staining solution and then placed directly on the freshly cut gel surface.

In general, suitable typings were obtained with samples which had been stored as hemolysates for less than 1 year at —80° C. In those cases where poor patterns were observed, generally represented by components migrating faster than normal, a fresh hemolysate was prepared using red cells stored in liquid nitrogen. In such cases no evidence of the fast components was observed.

Results

The results with respect to rare variants and polymorphisms are summarized in 3 tables. Table 2 summarizes the findings by tribe and village for those systems in which neither rare variants nor polymorphisms were encountered. Table 3 presents a similar summary for those systems in which rare variants but no well-known polymorphisms were encountered, as well as the findings for a "private" polymorphism of $ESA_{1,2,3}$. Table 4 presents data on systems in which certain well-known genetic polymorphisms were encountered, with or without rare variants. In all tables we indicate the frequency of 'no-types', patterns which despite repetition did not resolve into bands permitting typing. Although there is always the chance of a variant hidden amongst these, the usual explanation is deterioration of the sample in transit.

A "Private" Polymorphism of $ESA_{1,2,3}$ in the Macushi and Wapishana

Description of the Variant. When α-naphthol acetate is employed as the substrate, typically 3 regions of $ESA_{1,2,3}$ activity are seen in electrophoretograms of erythrocyte hemolysates; following Tashian and Shaw (1962) and Tashian (1965) these have been designated A_1, A_2 and A_3 (in this paper, $ESA_{1,2,3}$). All contain multiple components, as shown for the normal pattern in Figure 2a. This pattern closely resembles that described by Tashian and Shaw except that the B esterase activity is in greater proximity to the A_3 bands under the conditions we employed, probably due to the lower pH of the gel buffer in our screening technique. In our hands the resolution of these $ESA_{1,2,3}$ bands was greatly improved at this lower pH, especially when the gel was made of Electrostarch. As previously reported by Tashian (1969), fast moving artifact bands were observed in samples stored for long periods at —70° C. When such bands interfered with the reading of a gel it was usually possible to make a fresh hemolysate from a sample stored in liquid nitrogen.

Table 2. Systems in which no variants or polymorphisms were encountered

Tribe	Village	Systems											
		TF	PGM_2	CA_1	LDH	AK	PEPB	ICD	HGB A	HGB A_2	TPI	NP	Total
Macushi	AB	90	88	88	88	88	88	88	88	88	88	88	970
	CD	197	194	194	194	194	194	194	194	194	194	194	2137
	EFG	221	217	216	217	217	217	216	218	218	217	216	2390
	Total	508	499	498	499	499	499	498	500	500	499	498	5497
	All villages "no types"	1	0	0	0	0	0	0	0	0	0	0	1
	Grand total	509	499	498	499	499	499	498	500	500	499	498	5498
Wapishana	A	62	62	62	62	62	62	62	62	62	62	62	682
	B	64	64	64	64	64	64	64	64	64	64	64	704
	C	20	20	20	20	20	20	20	20	20	20	20	220
	D	41	41	41	41	41	41	41	41	41	41	41	451
	E	70	70	70	70	70	70	70	70	70	70	70	770
	F	32	32	32	32	32	32	32	32	32	32	32	352
	G	58	54	54	54	54	54	54	54	54	54	54	598
	H	42	42	42	42	42	42	42	42	42	42	42	462
	JK	85	84	84	84	84	84	84	84	84	84	84	925
	L	96	94	93	94	94	94	94	94	94	94	93	1034
	M	53	52	52	52	52	52	52	52	52	52	52	573
	Total	623	615	614	615	615	615	615	615	615	615	614	6771

Table 3. Systems in which only private variants but no well-known polymorphisms were encountered. Where more than one variant was encountered in a village, the numbers of each are given

Tribe	Village		Systems									Total
			CRPL	ALB	ESA	MDH	ADA	PEPA	PHI	CA_2	6-PGD	
Macushi (26)	AB	V	1	0	20	0	0	0	0	0	0	
		Total typed	90	90	88	88	88	88	88	88	88	796
	CD	V	0	0	0	0	0	0	0	0	2	
		Total typed	197	197	194	194	194	194	194	194	194	1752
	EFG	V	3	0	28[a]	1	0	0	0	0	0	
		Total typed	222	222	216	217	217	217	217	216	217	1961
	Total	V	1,3	0	48	1	0	0	0	0	2	
		Total typed	509	509	498	499	499	499	499	498	499	4509
Wapishana (27)	A	V	0	15	16[a]	0	0	15[b]	0	0	1	
		Total typed	62	62	62	62	62	62	62	62	62	558
	B	V	0	0	0	0	0	0	0	2	5	
		Total typed	64	64	64	64	64	64	64	64	64	576
	C	V	3	1	3	0	1	3	0	0	0	
		Total typed	20	20	20	20	20	20	20	20	20	180
	D	V	0	0	0	0	0	0	0	0	0	
		Total typed	41	41	41	41	41	41	41	41	41	369
	E	V	1	0	2	0	0	0	0	0	0	
		Total typed	70	70	70	70	70	70	70	70	70	630
	F	V	0	0	0	0	0	0	0	0	0	
		Total typed	32	32	32	32	32	32	32	32	32	288
	G	V	0	0	0	0	1	0	0	1	0	
		Total typed	57	58	54	54	54	54	54	54	54	493
	H	V	4	0	0	0	0	0	1	0	0	
		Total typed	42	42	42	42	42	42	42	42	42	378

JK	V	0	0	0	0	0	0	0	0	3	
	Total typed	85	85	84	84	84	84	84	84	84	758
L	V	1	3	1	0	0	2	0	0	2	
	Total typed	95	96	93	94	92	93	94	93	94	844
M	V	1	4[a]	6	0	0	0	0	0	1	
	Total typed	53	53	52	52	52	52	52	52	52	470
All villages	V	4,4,2	23	28	0	2	20	1	3	12	
	Total typed	621	623	614	615	613	614	615	614	615	5544
	"no types"	1	0	0	0	2[c]	0	0	0	0	
	total	622	623	614	615	615	614	615	614	615	

[a] Includes one homozygous individual
[b] Includes 3 homozygous individuals
[c] No activity

Table 4. Systems in which genetic polymorphisms of wide-spread occurrence were encountered, with or without the additional occurrence of private variants included in the present study

	Haptoglobin						PGM_1								Acid Phosphatase							
	1	1–2	2	0	Total	Hp^1	1	1–2	2	1–10	Total	PGM_1^1	PGM_1^2	PGM_1^{10MI}	B	AB	A	B–C	Total	ACP^A	ACP^B	ACP^C
Macushi (26)																						
AB	20	44	12	14	90	0.553	39	40	9	–	88	0.670			80	8	–	–	88		0.955	
CD	56	95	43	3	197	0.534	164	27	3	–	194	0.915			188	5	–	1	194	0.013	0.984	0.003
EFG	48	125	33	16	222	0.536	143	57	11	6	217	0.804	0.182	0.014	203	14	–	–	217		0.968	
Total	124	264	88	33	509	0.538	346	124	23	6	499	0.824	0.170	0.006	471	27	–	1	499	0.027	0.972	0.001
Wapishana (27)																						
A	11	38	13	–	62	0.484	29	31	2		62	0.718			51	9	2		62		0.895	
B	17	32	10	5	64	0.559	44	18	2		64	0.828			60	3	1		64		0.961	
C	2	13	5	–	20	0.425	11	9	–		20	0.775			19	1	–		20		0.975	
D	12	19	8	2	41	0.551	27	13	1		41	0.817			32	7	2		41		0.866	
E	9	22	38	1	70	0.290	22	36	12		70	0.571			66	4	–		70		0.971	
F	6	13	12	1	32	0.403	14	13	5		32	0.641			32	–	–		32		1.000	
G	5	28	24	1	58	0.333	43	9	2		54	0.880			45	8	1		54		0.907	
H	4	26	10	2	42	0.425	39	3	–		42	0.964			37	5	–		42		0.940	
JK	19	43	20	3	85	0.494	54	28	2		84	0.810			76	8	–		84		0.952	
L	35	43	18	–	96	0.589	58	32	4		94	0.787			85	9	–		94		0.952	
M	20	20	12	1	53	0.577	27	21	4		52	0.721			43	9	–		52		0.913	
Total	140	297	170	16	623	0.475	368	213	34		615	0.772			546	63	6		615		0.939	

	ESD					GALT								Total
	1	1–2	2	Total	ESD[1]	N	N+D	D	N+Wap.	Total	GALT[1]	GALT[D]	GALT[W1]	
Macushi (26)														
AB	40	38	10	88	0.670	77	11	–	–	88	0.938	0.062	–	442
CD	101	73	20	194	0.709	163	29	2	–	194	0.915	0.085	–	973
EFG	96	99	21	216	0.674	171	43	3	–	217	0.887	0.113	–	1089
Total	237	210	51	498	0.687	411	83	5	–	499	0.907	0.093	–	2504
Wapishana (27)														
A	35	20	7	62	0.726	58	4	–	–	62	0.968	0.032		310
B	38	26	–	64	0.797	40	21	3	–	64	0.789	0.211		320
C	8	11	1	20	0.675	16	3	1	–	20	0.875	0.125		100
D	21	17	3	41	0.720	34	7	–	–	41	0.915	0.085		205
E	51	16	3	70	0.843	59	10	1	–	70	0.914	0.086		350
F	20	6	6	32	0.719	27	5	–	–	32	0.922	0.078		160
G	38	16	–	54	0.852	51	2	–	1	54	0.972	0.019	0.009	274
H	25	17	–	42	0.798	30	12	–	–	42	0.857	0.143		210
JK	47	35	2	84	0.768	59	23	2	–	84	0.839	0.161		421
L	65	24	3	92	0.837	78	16	–	–	94	0.915	0.085		470
M	43	8	1	52	0.904	42	10	–	–	52	0.904	0.096		261
Total	391	196	26	613	0.798	494	113	7	1	615	0.896	0.103	0.001	3081

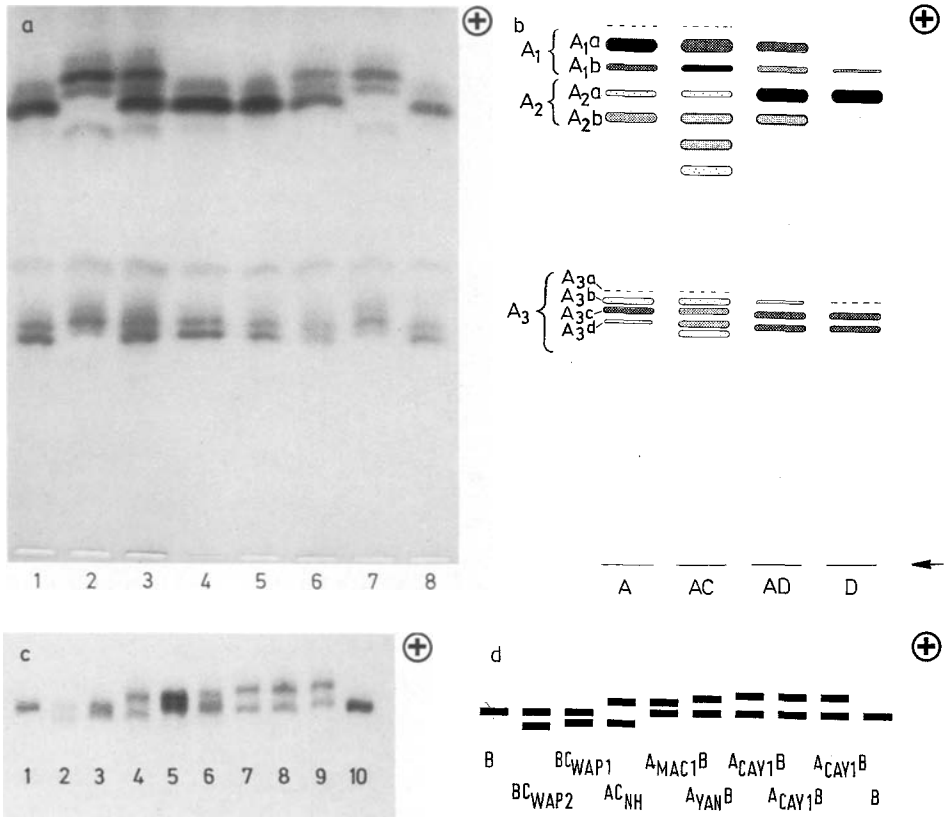

Fig. 2. a Starch gel stained with α-naphthyl acetate for ESA; wells 2 and 7 contained the normal type; wells 3 and 6 contained examples of the more common (heterozygous) variant type (AD); wells 1, 4, 5 and 8 contained examples of the less common (homozygous) variant type (D). Further explanation in text. **b** Diagram of the isozymes observed in the normal (A), the heterozygote for the $ESA_{1,2,3}$ variant (AD) and the homozygote for the $ESA_{1,2,3}$ variant (D). The AC variant of Tashian (1965) is also diagrammed for purposes of comparison. The Esterase B band, migrating between the $ESA_{1,2,3}$ 2 and $ESA_{1,2,3}$ 3 bands, is omitted from the diagram. The nomenclature employed is taken from Tashian (1969). **c** A study of Amerindian CRPL variants, using polyacrylamide gel electrophoresis; wells 1 and 10 contained normal ceruloplasmin B; well 2, $BC_{WAP\,2}$; well 3, $BC_{WAP\,1}$; well 4, $AC_{\text{New Haven}}$ standard; well 5, $BA_{MAC\,1}$; well 6, BA_{YAN}; well 7, Wapishana variant identified as $BA_{CAY\,1}$; well 8, Macushi variant identified as $BA_{CAY\,1}$; well 9, Cayapo variant illustrating $BA_{CAY\,1}$. Further explanation in text. **d** Diagram of the CRPL patterns presented in Figure 2c

Two kinds of atypical $ESA_{1,2,3}$ patterns were observed, one common, one rare. Both patterns are shown in Figure 2a. As can be seen from Figure 2a, the more common variant is characterized by additional, slower moving bands in the A_3 region. In addition the relative staining intensity of some of the normal bands is reduced or abolished. Specifically, there is observed a considerable reduction of the staining intensity of the A_1a band as well as the A_1b band. Concomitantly, there is a marked increase in the staining intensity of the A_2a band. Additional distinguishing features involve an apparent diminution of the A_2b band and a subtle

alteration of the activities and mobilities of the A_3 bands, characterized by an apparent loss of the A_3a band, a weakening of the A_3b band and an enhancement of the activity of the A_3c and A_3d bands. Also, the latter bands migrate slightly less rapidly towards the anode. Similar but further changes occur in the case of the less common variant. The A_1a and A_2b components appear to be completely lost and there is a dramatic increase of activity in the region of the A_2a band. The A_3 region remains unaltered relative to the more common variant. These changes are shown diagrammatically in Figure 2b. Such a finding is consistent with the concept of a shared subunit within the $ESA_{1,2,3}$ system. We interpret these complex changes as due to a mutation affecting this shared subunit, resulting in multiple additional components with altered (slower) electrophoretic mobility, one of which corresponds to that of the A_2a band, the other slightly slower than the A_3c and A_3d bands. However, the other slower-moving bands expected with the concept of a shared subunit are not seen.

The suggestion of a shared subunit was previously advanced by Tashian (1965) on the basis of an analysis of 3 isolated $ESA_{1,2,3}$ variants encountered in a screening of 4117 blood samples from diverse ethnic groups. Two of these were B types where additional faster-moving bands were observed and one was a C type where extra slower-moving components were seen. Our variant differs from the C-type variant Tashian described in the following respects: Both exhibit slower moving components and apparent enhancement of activity of some bands; however, in Tashian's variant the A_1b band became more active whereas in our variant the A_2a band became much more intense. Both variants showed a reduced A_2b band intensity. Presumably, in the case of Tashian's variant, the additional slow moving bands in the A_2 region represent an electrophoretic shift of some of the A_2 esterase protein. In the case of our variant only a diminution of A_2b activity was seen and no new bands were seen in the A_2 region in either of the patterns. Finally, as in the case of Tashian's variant, a shift in the relative intensities of some of the A_3 bands was also seen in the Amerindian variant. Specifically, the A_3b band diminishes and there is a concomitant increase in the activity of the A_3d.

A pertinent pedigree illustrating the inheritance of the Amerindian ESA variant in the Wapishana is shown in Figure 3. All data considered, the common variant pattern clearly represents the heterozygous state for a codominant gene which when homozygous results in the less common variant pattern. We will designate the variant patterns as $ESA_{1,2,3}\ AD_{MAC\,I}$ and $ESA_{1,2,3}\ D_{MAC\,I}$, and the responsible gene as $ESA_1^{D\,MAC\,I}$

Distribution of Variant in Macushi and Wapishana Villages. As shown in Table 3, the variant was observed in Wapishana as well as Macushi villages. In both tribes it has a very uneven frequency, ranging from 0 to 22.7% in the inhabitants of the Macushi villages and from 0 to 25.8% in the inhabitants of the Wapishana villages.

Absence of the ESA Polymorphism in Other Tribes. With the demonstration of an $ESA_{1,2,3}$ variant in polymorphic proportions in these two tribes, it was of some interest to check for its presence in such other tribes of South America as was possible from the material on hand. No example of the variant was found in 382 Makiritare, 232 Yanomama, 146 Piaroa, 404 Cayapo, 190 Krahó, and 112 Moro.

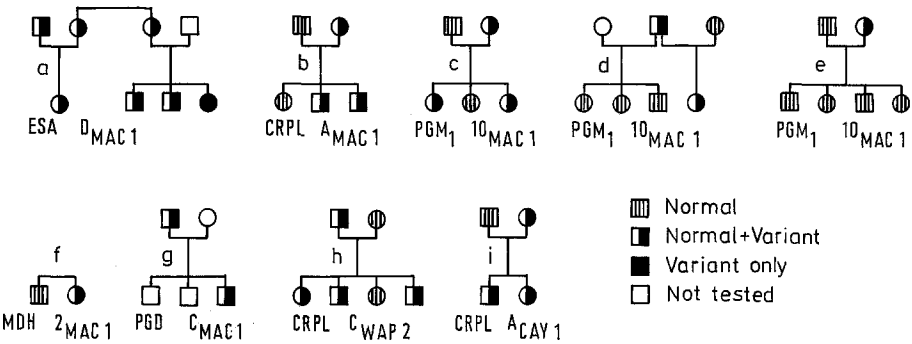

Fig. 3a—i. Nine pedigrees of variants encountered in the course of this study. **a** $ESA_{1,2,3}$ D variant of Macushi and Wapishana. **b** CRPL A_{MAC1} variant of Macushi. **c, d, e** PGM_1 10 variant of Macushi. **f** MDH_S2_{MAC1} variant of Macushi. **g** PGD C-type variant encountered in Macushi. **h** CRPL C_{WAP2} variant encountered in Wapishana. **i** CRPL A_{CAY1} in the Wapishana

The absence of the trait in the Makiritare is of especial interest. The Macushi, Pemon, and Makiritare are 3 Carib-speaking tribes in continuity with one another, the Pemon to the northwest of the Macushi, the Makiritare to the west of the Pemon. These tribes have traditionally maintained an active trade network (Thomas, 1973), with the attendant opportunities for gene flow.

Mestriner et al. (1976) have recently reported on a survey of 1070 persons from 8 South American Indian tribes with respect to the ESD polymorphism (Makiritare, Yanomama, Parakanan, Cayapo (Gorotire), Krahó, Moro, Caingang and Mapuche). They observed no variation in the $ESA_{1,2,3}$ isozyme bands, which are easily visualized in the ESD preparations. We have established that the ESA polymorphism can usually be classified with the electrophoretic conditions employed by Mestriner et al. (1976). There is thus this additional evidence for the absence of the $ESA_{1,2,3}$ polymorphism in other tribes. Incidentally, the Makiritare and Yanomama studied by Mestriner et al. (1976) are from different villages than those examined by ourselves; whereas the Krahó and Moro samples are essentially the same (but were examined under different electrophoretic conditions). Finally, the 163 Gorotire of Mestriner et al. (1976) are included in our sample of 404 Cayapo (again examined under different electrophoretic conditions).

Description of the Rare Variants Encountered

A total of 15 rare variants has been encountered in the 25 systems screened. Unlike most of the tribes we have previously studied, there is evidence of significant admixture with non-Indians in these 2 tribes (cf. Neel et al., in press). Accordingly, in this presentation we will attempt to indicate those electromorphs which *might* have been introduced and those which should be regarded as autochthonous.

Macushi Variants

CRPL. Two CRPL patterns characterized by a fast-moving (A-type) band in addition to the normal band were seen. Three individuals from one family

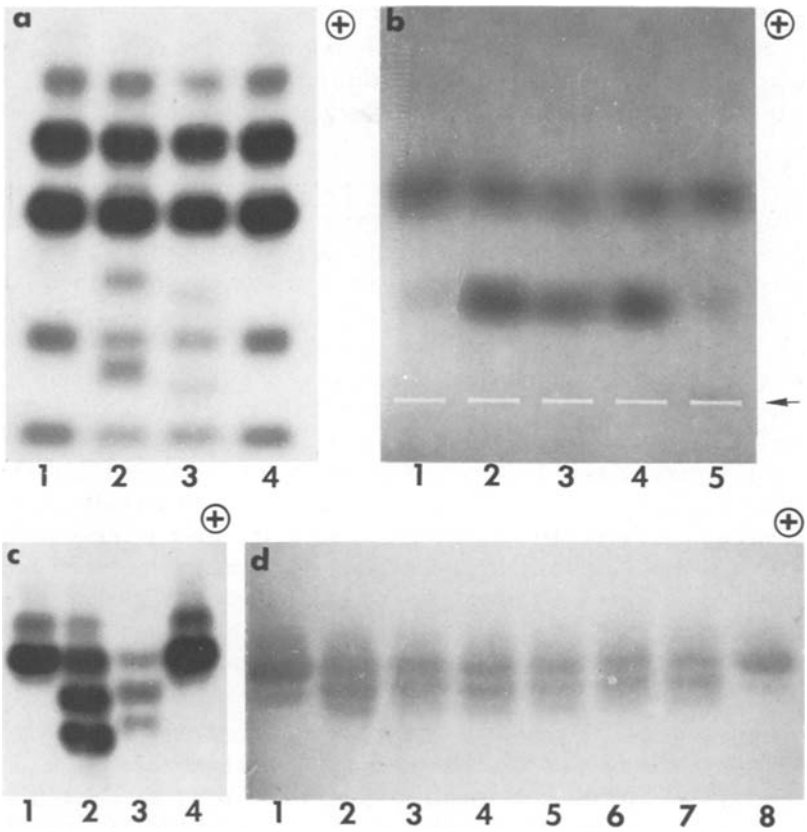

Fig. 4. **a** Starch gel pattern of the PGM_1 1—10 variant (well 3) compared with a PGM_1 1—2 (well 2) and PGM_1 1 (wells 1 and 4). **b** ACP_1 patterns obtained with starch gel and phenolphthalein diphosphate as stain. Wells 1 and 5 contained normal type B; wells 2 and 4 contained BC standards from two different Caucasian controls; well 3 contained the Macushi sample. **c** Comparison of the Macushi MDH_S 1—2_{MAC1} variant (well 2) with the normal pattern (wells 1 and 4) and the MDH_S 1—2 variant of Davidson and Cortner (1967) (well 3, pattern from fibroblasts). **d** Electrophoretic comparisons of the PGD type AC variants. Wells 1 and 8, normal type A; well 2, Guaymi PGD AC; well 3, Jamaican Negro PGD AC for comparison purposes; well 4, Makiritare PGD AC; well 5, Cayapo PGD AC; well 6, Wapishana PGD AC; well 7, Macushi PGD AC

exhibited the pattern illustrated in Figure 2c, well 5; the pedigree of the 3 persons involved is shown in Figure 3b. A single individual from another village exhibited the pattern shown in Figure 2c, well 8; no known relative of his is included in the sample. For purposes of comparison we also show in Figure 2c the two other fast moving variants we have encountered to date in Amerindians, in the Cayapo (Tanis et al., 1973) and in the Yanomama (Weitkamp et al., 1972). Figure 2d is a diagrammatic representation of the findings. The variant encountered in a single Macushi is electrophoretically identical with the Cayapo variant and we designate it as A_{CAY1}. The other Macushi variant clearly differs from both the Cayapo and Yanomama variants. We shall designate this latter variant A_{MAC1},

the pattern as $BA_{MAC\,1}$, and the gene as $CRPL^{A\,MAC\,1}$. (In Figure 2c, well 9, both the normal and the $A_{CAY\,1}$ band migrate slightly faster than the corresponding bands in wells 7 and 8, but the distance between the two bands is the same for all 3 specimens. In other runs this sample has migrated identically with those in wells 7 and 8.)

PGM_1. Six examples of a PGM_1 electrophoretic variant were observed in a single Macushi village. The variant pattern occurred in all cases in combination with the normal type 1 pattern. The phenotype is compared in Figure 4a with a typical 1—2 pattern; the variant b and d bands are both shifted towards the cathode in comparison with the type 2 phenotype. Pedigree data on the individuals are given in Figures 3c, d, and e. A comparison of the Macushi variant with the variants reported to date suggests it migrates electrophoretically in a manner most similar to that of the PGM 1—4 variant of Hopkinson and Harris (1966); a sample of 1—4 was not available for comparison. However, based on the relative staining intensities of the components of 1—4 as reported by these authors we believe our PGM_1 variant is different. As can be seen in Figure 4a both of the variant components stain moderately strongly and are of about the same intensity. This staining pattern is quite different from the PGM 1—4 pattern, in which the variant bands are quite weak, particularly the more anodal component. We therefore believe this represents a new variant of the PGM_1 system which we propose to designate as PGM_1 $10_{MAC\,1}$, the pattern observed being PGM_1 $1—10_{MAC\,1}$.

ACP. During the course of this survey an ACP type BC was observed in a single Macushi individual. Figure 4b contains a comparison of this sample with 2 BC phenotypes from Caucasians. Both alleged parents of this individual lacked the variant but there was a parentage exclusion on the basis of the MNSs and the Rh blood groups. Given this fact plus the occurrence of the ACP^C allele in approximately 8% of Caucasians and 2% of Negroes (and the evidence to be presented later of Negro-Caucasian admixture with the Macushi), we believe the conservative course is to treat this as an introduced (non-Indian) variant.

MDH_S. A single Macushi individual exhibited a variant pattern of MDH_S; a comparison of this pattern with the normal pattern is shown in Figure 4c. The additional slow-moving bands represent the hetero- and homopolymers in this dimeric system (Hopkinson et al., 1976). The only other relative examined was a brother who had a normal pattern (Fig. 3f). Although the pattern of the Macushi variant has features in common with the MDH_S 1—2 pattern previously reported to occur at a very low frequency in Negroid, Mongoloid and Caucasoid populations (Davidson and Cortner, 1967; Leaky et al., 1972) a direct comparison with an MDH_S 1—2 phenotype in cultured fibroblasts kindly supplied by Dr. R. G. Davidson revealed sufficient differences that we believe this to be a new variant (Fig. 4c). The individual in question was type 0, Kell-negative, Gm (a+, x+, g+), i.e., no evidence of non-Indian ancestry. While the possibility of the introduction of this variant by neo-Brazilians cannot be rigorously excluded, the odds against this are high and we believe it more likely to be autochthonous. Because of the general resemblance of the phenotype to a 1—2, we designate the variant as MDH $1—2_{MAC\,1}$ and the responsible allele as $MDH^{2\,MAC\,1}$.

PGD. Variants of this system were detected in two related Macushi (pedigree in Fig. 3g). The patterns of these two individuals have been compared with the PGD AC phenotype present in other Indian tribes (see below) and an AC from a Jamaican Negro and found to be identical. A sample from one of these two Macushi is included in the comparison run shown in Figure 4d, well 1. All the AC patterns appear identical. The reader is referred to the appropriate section in the presentation of the results in the Wapishana for a more detailed treatment of this variant.

Wapishana Variants

CRPL. Two different CRPL variants characterized by slow-moving bands have been encountered in the Wapishana. The first variant to be observed, with the faster-moving of the variant bands (Fig. 2c, well 3), was encountered in two individuals living in different villages, neither with recorded relatives in the sample. The second variant observed, with the slower-moving of the slow variant bands, appeared in the patterns of 4 related individuals; the electrophoretic pattern is shown in Figure 2c, well 2, and the pedigree in Figure 3h. No other slow moving variants have been encountered to date in Amerindians; but for purposes of comparison we include an $AC_{\text{New Haven}}$ standard (Shokeir and Shreffler, 1970) in Figure 2c, well 4. The Wapishana C variants are clearly different from each other but the faster has a mobility indistinguishable from $C_{\text{New Haven}}$. This variant was encountered in 1.7% of a sample of 1969 American, Nigerian, and Haitian Blacks (Shokeir and Shreffler, 1970). We are aware of no reports of this variant in Caucasians. Interestingly, the carrier father shown in Figure 3h was ABO type A_2, Kell-negative, Gm fb, i.e., there is strong evidence for *Caucasian* ancestry. While the possibility cannot be rigorously excluded, we consider it unlikely this variant has been introduced by admixture. We shall therefore designate this faster variant of CRPL C as CRPL C_{WAP1}, the pattern as BC_{WAP1}, and the allele presumed to be responible for the variant, $CRPL^{C\,\text{WAP}\,1}$. Likewise, we designate the second, the slower, variant as CRPL $C_{\text{WAP}\,2}$, the pattern as $BC_{\text{WAP}\,2}$, and the responsible allele as $CRPL^{C\,\text{WAP}\,2}$. The relative electrophoretic mobilities of all these variants are diagrammed in Figure 2d.

In addition we have detected a fast (A-type) variant of CRPL in the Wapishana. This variant pattern has been compared with, and found to be indistinguishable from, the $BA_{\text{CAY}\,1}$ pattern (confer Figure 2c, well 7); consequently it will be designated as such. The trait has been observed in 4 individuals, 3 of whom were related; the fourth individual was from another village and no relatives were included in our sample. The relevant pedigree information is given in Figure 3i.

ALB. We have previously reported the occurrence in the Wapishana of an albumin variant in 15 of 62 persons in the only Wapishana village tested up to that time (Tanis et al., 1973). By a number of criteria, this variant was indistinguishable from a variant previously reported by our group, albumin Makú, found in several Makú Indians living among the Yanomama (Weitkamp and Chagnon, 1968). Following our convention of designating variants with the same electrophoretic characteristics by the tribe of first discovery, the variant encountered

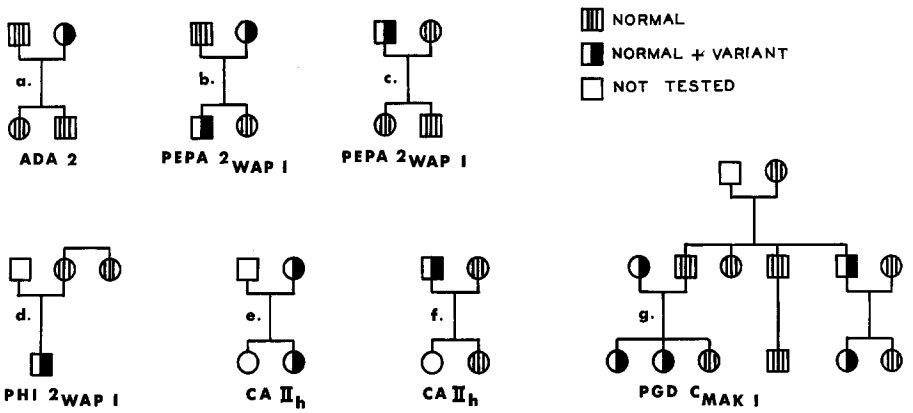

Fig. 5a—g. Six pedigrees of variants encountered in the course of the study. **a** The ADA type 2 variant encountered in the Wapishana. **b, c** The PEPA $2_{WAP\,1}$ pattern encountered in the Wapishana. **d** PHI $2_{WAP\,1}$ in the Wapishana. **e, f** Two pedigrees of the CA_{II} variant of the Wapishana. **g** PGD C variant of the Wapishana

in the Wapishana must be designated albumin Makú, but the possibility of an independent origin of the Wapishana variant of course cannot be excluded. Additional examples of this variant, at low frequencies, have now been encountered in 3 other Wapishana villages (cf. Table 3). Numerous pedigrees indicate the usual pattern of codominant inheritance (cf. Tanis et al., 1973).

ADA. Two unrelated Wapishana individuals from separate villages had identical ADA variant patterns characterized by an additional slow-moving band, as shown in Figure 6a. We have compared one of these samples with a standard ADA 1—2; it appears electrophoretically identical (Fig. 6a). Accordingly, we have designated the variant as ADA 2. Pedigree information for one of these two individuals is given in Figure 5a; in the other case no known relatives were contained in our sample. A variant of this type occurs in polymorphic proportions in a wide variety of human populations (Spencer, Hopkinson and Harris, 1968). Once again, given the evidence of admixture with Caucasians and Negroes in this tribe (Neel et al., in press), we believe the conservative course is to treat this as an introduced variant.

PEPA. Previously we have reported the occurrence of a variant of PEPA in 13 members of a sample of 60 individuals from the same Wapishana village (Tanis et al., 1973). (There was a numerical error in the original report; the correct figure is 15/62.) On the basis of comparisons with the literature, we indicated that the variant gel pattern most closely resembled the type 1—2 reported by Lewis and Harris (1967). We have now screened an additional 552 Wapishana and detected an additional 5 individuals, in 2 villages, who are heterozygous for the responsible gene. Thus the high frequency of the variant pattern we initially reported is not typical of the tribe as a whole and, in fact, the variant is not present in most of the villages sampled.

Figure 6b consists of a comparison of the Wapishana variant with examples of the 1—2 phenotype which exists at a polymorphic frequency in Negro populations

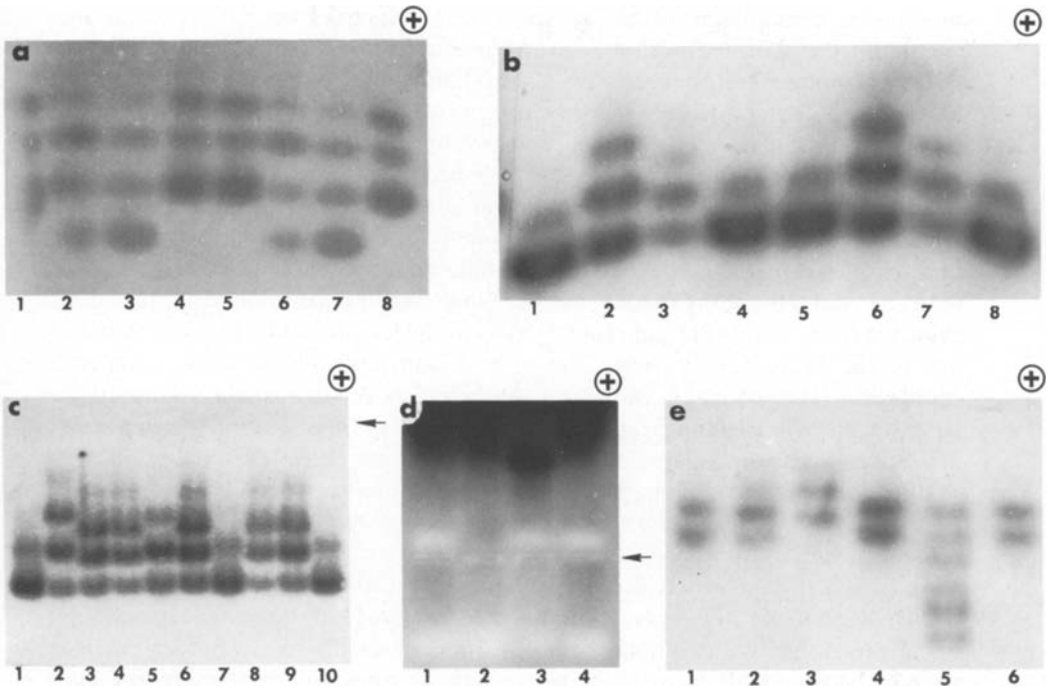

Fig. 6. a A comparison of the Wapishana ADA variant (well 2) with the normal type 1 (wells 1 and 4) and a type 1—2 (well 3). Wells 5—8 repeat the comparison. See text. **b** The Wapishana PEPA 1—2 variant (well 3) compared with a Negro type 1—2 (well 2) and the common type 1 (wells 1 and 4). Wells 5—8 repeat the comparison. See text. **c** Polyacrylamide gel electrophoresis comparison of PHI variants. Arrow indicates origin. Wells 1, 7 and 10 contained the normal type 1; well 2 was a PHI type 1—3 standard added for comparative purposes (we thank Dr. Eloise Giblett for the sample); wells 3 and 4 contained the Krahó variant, PHI $1—2_{CAY}$; well 5 contained the Wapishana variant, PHI $1—3_{WAP}$; wells 6, 8 and 9 contained the Cayapo variant PHI $1—2_{CAY}$. **d** Starch gel comparison of the Wapishana CA_{II} variant (wells 1 and 4) with normal (well 2) and a $CA_{II}2$ standard from an American Negro (well 3). The gel was stained with fluorescein diacetate. Arrow indicates origin. The dark area is produced by the presence of hemoglobin A. **e** Electrophoretic comparison of the Wapishana GALT variant (well 5) with normal (wells 1, 4 and 6) and with persons heterozygous (well 2) and homozygous (well 3) for the Duarte variant

(Lewis and Harris, 1967). The variant band in the "common" 1—2 phenotypic pattern is characterized by a more rapid migration towards the anode than the variant band of the Indian sample. In addition to the obvious difference in electrophoretic mobility, we note that the Indian variant is much less stable than the common type 2 variant when stored at —70° C or in liquid nitrogen. This new variant has been designated PEP 2_{WAP1} and the pattern PEPA $1—2_{WAP1}$. Pedigree information on the additional variants is contained in Fig. 5b and c; 2 of the 5 new examples of the variant had no known relative in the sample. The allele is termed $PEPA^{2\,WAP1}$.

PHI. A single Wapishana exhibited an electrophoretic variant of PHI on starch gel electrophoresis. This person had only two known relatives (Fig. 5d). Figure 6c

presents a comparison of this pattern with examples of the PHI variants encountered in 2 other South American tribes, the Cayapo and Krahó, employing polyacrylamide electrophoresis (7.1% acrylamide, pH 8.5, buffer 0.037M Tris, glycine). The comparison provides strong evidence that the Wapishana variant pattern is different from the variant patterns seen in the Krahó and Cayapo tribes; details on the latter have been published (Tanis et al., 1973). We interpret Figure 6c (as well as runs on starch gel) as showing that the Krahó and Cayapo variant patterns are identical. Furthermore, they differ from the Wapishana sample in that they contain two intermediate bands which migrate more rapidly than the corresponding bands of the Wapishana variant. There is also clear evidence for a weakly staining fourth band in the Krahó and Cayapo pattern, but not in the Wapishana pattern. For comparison purposes we have included a standard PHI type 1—3. Based on the behavior relative to this standard we consider the Wapishana variant to resemble a type 1—3 pattern more closely than any other. This resemblance is somewhat less on starch gel electrophoresis. We shall for the present designate these two different patterns as PHI $1—3_{WAP\,I}$ and PHI $1—2_{CAY\,I}$; the latter designation also includes the Krahó variant, which will be treated in detail elsewhere.

CA_{II}. In 3 Wapishana individuals, 2 of whom are related as mother and daughter, a second carbonic anhydrase II band was observed just anodal to the origin. Pedigrees are shown in Figure 5e and f. In all cases the enzymatic activity was approximately equally distributed between the 2 bands; this can be seen in Figure 6d. A similar electrophoretic variant of human CA_{II} has been previously reported by Moore and her colleagues (1971), who have determined that it occurs at polymorphic levels in Negroes. A comparison of the Wapishana variant with an example of this CA_{II} 2 variant of Negroes is also shown in Figure 6d. Under these electrophoretic conditions there is no apparent difference between the Wapishana variant and the Negro CA_{II} 2 variant, and in view of the evidence for Negro admixture with the Wapishana, to be presented in the following paper, we consider this variant to have been introduced, although the possibility of a local origin of course cannot be excluded. The Gm serotypes of the persons in these pedigrees were axg, axg, and agfb, the latter suggestive of Caucasian (but not Negro) admixture.

GALT. A variant indistinguishable from the Duarte variant of GALT (Beutler et al., 1965) reached polymorphic proportions in this tribe (see below). In addition, a single individual exhibited a complex pattern of 4 additional slowly-moving bands (Fig. 6e). For comparative purposes examples of the heterozygous and homozygous Duarte variant are also shown in Figure 6e. It is noteworthy that whereas in the Duarte variant, presumably due to a single amino acid substitution, there are only 2 additional bands, in this variant, designated $GALT_{WAP\,I}$, there are, as noted, 4 clearly delimited, additional slowly-moving bands. This enzyme is composed of two identical subunits (Dale and Popják, 1976). Sample denaturation seems an unlikely explanation. Although the variant is most likely due to a single amino acid substitution, we have no satisfactory explanation for the occurrence of 4 additional bands. Unfortunately, this individual had no known relatives in our sample. To our knowledge no other human variants of this system have been reported.

PGD. Twelve examples of a PGD variant were seen in the Wapishana. On the basis of comparisons with gel patterns in the literature (Parr, 1966), this variant most probably represents the PGD AC phenotype. We have by now detected a similar pattern in 3 other tribes [Macushi (see above), Cayapo (unpublished), and Makiritare (Weitkamp and Neel, 1970)]. A comparison of the phenotypic patterns is provided in Figure 4d. Based on this comparison there appears to be no tangible difference between examples of this variant as seen in the 4 tribes. We originally (mistakenly) designated this variant as the B phenotype (Weitkamp and Neel, 1970). However, based on the widely accepted nomenclature of Parr (1966), this variant would most properly be designated as PGD C. Since it was first ascertained in the Makiritare Indians, we will designate the variant phenotype as PGD $AC_{MAK\,1}$ and the allele as $PGD^{C\,MAK\,1}$. Seven of the involved persons had no known relatives in the sample; the pedigrees of the remaining 5 persons are shown in Figure 5g.

Since the C phenotype occurs at low frequencies in both Caucasians and Negroes, the question arises whether the responsible allele has been introduced into the Wapishana (and Macushi, see above). The occurrence of the gene in the Cayapo and Makiritare, where there is no evidence of admixture, makes it clear this allele may be an Indian variant. However, there is in this connection a different type of problem. The frequencies are so low in the Cayapo and Makiritare (who are widely separated) that we believe separate origins of the phenotype to be as probable as spread from a common source. If the former hypothesis is espoused, then the case for introduction of the gene into the Macushi and Wapishana by neo-Brazilians is at least as likely as autochthonous origin.

Data on Genetic Polymorphisms of these 25 Systems

Genetic polymorphisms electrophoretically indistinguishable from well-known genetic polymorphisms were encountered in 5 of these 25 systems, namely, ACP_1, HP, PGM_1, ESD, and GALT. Phenotypes and gene frequencies are given in Table 4, together with data on rare variants (if encountered) for these systems.

Discussion

The interpretation of the data presented in this paper is highly dependent on the data on the common genetic polymorphisms to be developed in the following paper, and we shall delay most of the discussion for that paper. Accordingly, we limit our comments to the following:

The Tribe of Origin of the ESA Polymorphism. The highest village allele frequency of the $ESA_{1,2,3}$ polymorphism in the Macushi is 0.114 in 88 persons in village 26AB; the highest such frequency for the Wapishana is 0.137 in 62 persons in village 27A. For the entire sample of Macushi, the gene frequency is 0.049 whereas for the Wapishana sample, the corresponding figure is 0.024. It is unfortunate that the Macushi sample is restricted to only 3 villages from a limited part of the tribal distribution. The difference in gene frequency in these 3 Macushi

villages is noteworthy, the gene being absent in village 26CD but at frequencies of 0.114 and 0.067 in the other 2 villages. However, equally large differences between villages situated relatively close to one another were observed in the case of the private polymorphism of albumin observed in the Yanomama (Tanis et al., 1974); such differences can be attributed to the origin of a typical Indian village from a relatively few lineages, i.e., founder effect.

As noted earlier, we have collected data concerning recent admixture between the 2 tribes: it is a reasonable expectation that if the source of the gene is the Macushi, then the gene frequency should be higher among Macushi and their descendants residing among the Wapishana (and now nominally Wapishana) than among the remaining Wapishana. Conversely, if the source of the gene were the Wapishana, the gene should be observed in a relatively high frequency among the Wapishana and their descendants residing among the Macushi (and now nominally Macushi). From Table 1 we note that 47 Wapishana or Wapishana/Macushi were identified within the Macushi sample; only 1 of these carried the ESA_1^D allele. There were 72 known Macushi or Wapishana/Macushi within the Wapishana sample; only 1 of these carried the ESA_1^D allele. Although simple statistical tests cannot be performed because of the biological relationships of these people, there is certainly no evidence of the transmission of the gene from one group to another and, in fact, these migrants inexplicably, in both instances, have a lower gene frequency than the tribal average. We will return to this question of the origins of the gene in the following paper.

The Frequency of Rare Variants in the 2 Tribes. We have arbitrarily defined a rare variant as one present in less than 2% of a population. By definition, then, we must in this treatment of rare variants in these 2 tribes exclude the polymorphism of $ESA_{1,2,3}$ from consideration. However, when, eventually, we turn to a consideration of the results of all of our studies of Indian tribes for rare variants, the $ESA_{1,2,3}$ polymorphism would be included, since in the total material less than 2% of the population carry the variant. With this convention, on the basis of 12510 determinations, the frequency of rare variants in the Macushi is 1.1/1000 determinations, whereas, on the basis of 15396 determinations, the corresponding figure for the Wapishana is 4.7/1000. These figures may be compared with the previously published average for 6 Indian tribes, of 3.2/1000 (Tanis et al., 1973). Unfortunately, conventional statistical tests cannot be applied to the question of the significance of these differences. In subsequent papers we will be using these and additional data for direct and indirect estimates of mutation rates and in a search for evidences of selection. We have indicated that 3 of the 15 rare variants encountered in these 2 tribes may have been introduced (a C-type variant of ACP in the Macushi and a 2-type variant of ADA and a 2-type variant of CA_{II} in the Wapishana). In the companion paper, we will estimate the amount of admixture with neo-Brazilians represented in these 2 tribes, and attempt an appropriate adjustment of the frequency of rare variants.

In the *Introduction*, we mentioned the interest in determining whether the Macushi and Wapishana possessed the "private" polymorphism of albumin observed in the nearby Yanomama, who at one time may even have been contiguous to the ancestors of the present-day Wapishana. The fact that they do

not strengthens the case for the remarkable isolation of the Yanomama throughout much of their existence.

The Segregation Ratios for the Presumed Variants. When a variant occurs in polymorphic proportions, there is seldom doubt as to its genetic basis. With the very rare variants, the possibility of confusion with a persistent artifact always exists. Figures 3 and 5 have presented the available pedigree data on the rare variants. Among the offspring of a mating of normal with a presumed heterozygote, given our population sampling procedures, expectation for affected children is 50%; the ratio observed was 14 : 15. These very limited data thus contain no suggestion that technical artifacts are contributing to the apparent variants.

The Strange Case of Village 27A. The term private polymorphism can, of course, be extended to the village level. With this usage, Wapishana village 27A stands out as exhibiting 3 private polymorphisms of the 25 systems examined. Given the fact that at the village level a private polymorphism may be accounted for by the presence of a particular gene in a few extended lineages, it is difficult to attach any particular significance to this at this time. Elsewhere we have emphasized how in general in the peopling of the Americas, new tribes arose from old by a budding-off (non-random sampling) process (Neel, 1967, 1973). It is clear that should an off-shoot of the Wapishana include village 27A, the gene frequencies of the new tribe would for these 3 traits exhibit a major departure from those of the old, simply by virtue of stochastic events.

Implications of these Data for Mutation and Selection. One of the long-range objectives of this program of study of the Amerindian is to provide a body of data suitable for both direct and indirect estimates of the rate at which mutation results in electrophoretically detectable changes in proteins and for a search for evidence of selective forces. In this undertaking, in which the tribe is the unit of study, the primary effort is directed at estimating the number of different variants present in the tribe at the loci under consideration and the numerical frequency of each. Earlier we have mentioned the extensive admixture of the Wapishana with other Indian tribes and neo-Brazilians, who, however, as we shall show in the next paper, make only a 5 or 6% contribution to the tribal gene pool. While such admixture does not invalidate the use of the Wapishana in our effort to generate an estimate of the average frequency of rare variants among Amerindian tribes in general, it does preclude the use of the tribe in any future effort to estimate an average mutation rate on the basis of the rare variants present in the tribe, since the tribe no longer defines the population in which the variants arose.

The Gene Frequencies on the 5 Polymorphisms of these Systems. The gene frequencies for the 5-biallelic polymorphisms for which data are given in this paper are quite similar in the 2 tribes, as might be expected in view of the evidence which has been developed for admixture between the 2 tribes. With respect to other Amerindians of South America, the gene frequencies for HP^1, PGM_1^1, ACP^A, and ESD^1 are all within the range reported for Amerindian tribes (cf. Mestriner et al., 1976, Salzano et al., in press). The presence of a Duarte-like variant of galactose-1-phosphate uridyltransferase in Amerindians was first

recognized in 2 of 663 Yanomama tested by Weitkamp and Neel and reported in 1972. We subsequently discontinued but have now (1974) resumed testing for variants of this system, using the method of Sun et al. (1974). Not only does the variant appear in polymorphic proportions in the 2 tribes covered in this paper but also in several other tribes which we have now tested: Krahó, 0/191; Cayapo, 11/396; Moro, 0/114; Piaroa, 35 (3 homozygotes)/146; and Makiritare, 26 (1 homozygote)/388. We have also tested an additional 274 Yanomama (from other villages than the original sample), finding 10 heterozygotes.

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