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Absence of the Diego Antigen, a Genetic Characteristic of Early Immigrants to South America

Abstract. The Diego blood group is an exclusive Mongoloid gene marker, although it is not present in all Mongoloid populations. The absence of the gene in Waica Indians and its very low frequencies in Warrau and Yaruro Indians of South America suggest that it represents a genetic characteristic of Marginal American Indians. Since Marginal Indians are considered to be early comers to the New World, we suggest that Diego-negative populations were the first to arrive and to extend throughout South America, while the Diego-positive tribes came later.

The Diego blood group antigen was first discovered in a Venezuelan family of Carib Indian ancestry (*1*). Subsequently it proved to be an exclusive Mongoloid gene-marker, which is not present in Caucasoids and Negroids. This evidence, however, does not imply that the gene is carried by all Mongoloid populations or that it is carried by them in a more or less constant frequency. On the contrary, studies during the last 6 years have shown that there exist populations which, while classified as Mongoloid, do not carry the gene at all—for example, the Oceanic populations, the Eskimo, and several Amerindian tribes (*2*).

In a recent review of all the populations examined for this antigen, it was observed that populations with related languages tend to show similar Diego frequencies. One of the best documented cases studied is that of three groups of Venezuelan Cariban tribes which showed a Di (a +) range between 20 and 34 percent (mean 28 percent), in spite of being separated from each other by some 3000 years (30 m.c.) in time and over a distance of 1500 km (*2*, *3*). Thus, linguistic affiliation, together with several specific culture aspects of the various tribes, has proved to be useful in predicting the Diego frequencies in Indian populations. Since the identification of extreme culture types is less complicated than that of intermediate ones, we directed our studies toward those tribes of South America which are conventionally classified as Marginal. Combining the physical anthropologists' notion of Marginal Indians with that of the cultural anthropologists, we understand here as being Marginal those aborigines of South America who are predominantly dolichocephalic, of medium or stocky build, with varying degrees of Mongoloid features, nonagricultural, with little developed technology, and a simple social structure based on kinship rather than on class. The purpose of our study was to find out whether Diego antigen frequencies would show marked differences between Indian populations of very different culture types.

Last April we had an opportunity to study the Waica Indians of southern Venezuela, whose identification as Marginals can hardly be doubted (*4*). As a subtribe of some 10,000 individuals, they belong to the Yanoama, who also include the Sanemá, Samatari, Kasapare, Surára, and Pakidái of southern Venezuela and northwestern Brazil; these various languages form a linguistic stock of 6 to 35 m.c. of internal separation.

Without going into the details of the results of antigen analysis of the ten blood group systems, we would like to report here the absence of Diego in 142 samples. They were taken from less closely related individuals living in six settlements within an extensive area. We consider, therefore, that this sample is a genetic representative of the entire Waica population and, in consequence, that it rules out the possibility that the Diego result is due to gene drift.

This finding is considered to be of major importance, because it raises the figure of Diego-negative Marginal tribes of Venezuela to the number of three—that is, Warrau, Yaruro, and Waica (Table 1).

The Warrau (*5*) occupy the Orinoco Delta and the adjacent swampy regions of the coast of British Guiana (latitude, 8°–10°N; longitude, 59°–62°W). Two different subtribes were tested, one of them a peripheral community, called Guayo, in which some admixture with the neighbouring Indian tribes was expected to have occurred (Carib, Arawak). The other one, the Winikina, live in the center of the Orinoco Delta, where they have been living in complete isolation until very recently. The Guayo-Warrau showed a Di^a gene frequency of 1.9 percent, and the Winikina-Warrau were negative.

The Yaruro (*5*) Indians inhabit the savannahs or *llanos* of southwestern Venezuela (7°N, 68°W). They have been living in this alternately desert-like and flood-covered territory for many centuries, and there are reasons to assume that they took possession of it long before the discovery of America. From 102 samples tested, only five demonstrated the Di^a, of which four were derived from 11 samples, taken from a single band. This indicates that they do not represent actually a homogenous pool of Di^a genes.

For the reasons mentioned above, we suggest that the Warrau as well as the Yaruro were Diego-negative originally, and that their low Di^a frequencies are due to admixture with the neighbouring tribes who exhibit a relatively high frequency of the gene.

Contrasting with the absence of the Diego gene among Marginals are the elevated frequencies of 10 to 45 percent of Diego among peoples of a higher culture level and different linguistic affiliation—that is, Saliva, Arawak, Tupí, Carib, Chibcha, Quechua, and Aymara—and who, according to their culture and physical type, fit into the scheme of Coon *et al.* (*6*), as either Tropical Forest Indians or Central American Indians. Exceptions, which expectedly have come up, were discussed by the authors in the case of the Tunebo, Irapa, Colla, and Omaguaca

Table 1. Distribution of the Di^a genotype in Marginal Indian tribes.

Tribes	No. tested	Phenotypes (%)		Genotypes (%)	
		Di (a+)	Di (a−)	Di^a	Di
Winikina-Warrau	72	0.00	100.00	0.00	100.00
Guayo-Warrau	81	3.69	96.31	1.9	98.1
Yaruro	102	4.91	95.09	2.49	97.51
Waica	142	0.00	100.00	0.00	100.00

(*7*), but in each of these cases sufficient cultural and genetic evidence could be accumulated to show that discrepancies in the relationship between Diego gene frequencies and culture development were due to acculturation rather than to gene drift or natural selection. For instance, the Colla and Omaguaca, who adopted the Quechua language during the Spanish conquest, showed 3 percent of Di (a +) while the true Quechua tribe tested exhibited 25 percent. Tunebo, although linguistically classified as Chibchan, exhibited an incipient agriculture, and their physical features differ from other Chibcha Indians. The Diego frequency was only 1 percent for the Tunebo, while two other Chibcha tribes tested, Ica and Páez, demonstrated 41 and 31 percent, respectively. The Irapa, a Yupa subtribe which in turn is of Cariban affiliation, exhibited 2 percent of Di (a +), and four other Yupa subtribes tested had a range from 21 to 34 percent; also, the Rh and MNSs of the Irapa were statistically different from the other Yupa subtribes, while similar to the Southern tribe (Dobokubi) which showed a negative incidence of Diego (*8*). We are not proposing, of course, that a gene is to be made responsible for a certain culture pattern. However, from a parallel study of cultural characteristics and Diego frequencies, it is becoming gradually clear that different culture types go along with different genetic constitutions. In particular, Marginal Indians differ in both cultural and genetic aspects from the Tropical Forest Indians and the Central American Indians. It is obvious that this observation, if substantiated by future research, will be of great importance for the historical reconstruction of the peopling of America. At the present stage of Diego research, we propose that the Diego-negative peoples represent an early wave of immigrants in South America, and that most of them can still be identified by a Marginal type of culture. They were possibly followed by Diego-positive peoples, whose earlier waves would have had a better chance of interbreeding with the Diego-negative tribes than their later ones, thereby causing the different frequency ranges (*9*).

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Effect of Deuteration of N—CH_3 Group on Potency and Enzymatic N-Demethylation of Morphine

Abstract. Substitution of deuterium for the N-methyl hydrogens of morphine produced a significant reduction in the potency and lethality of morphine in mice regardless of the route of administration. There was no effect on the time of onset, maximal effect, or duration of action. N-demethylation by rat liver microsomal enzymes was characterized by a smaller reaction rate constant, a higher energy of activation, and a larger Michaelis constant with respect to the deuterated morphine. These findings indicated that deuteration of the N-methyl group of morphine not only caused reduction in potency, but also a reduction in the rate of oxidative N-demethylation, and a distinct weakening of the binding to the enzyme active centers.

The role of demethylation in the action and metabolism of morphine-like analgesics has been the subject of much recent work. Beckett *et al.* (*1*) postulate that N-dealkylation at the central receptor site is the initial reaction in the production of analgesia, and Axelrod (*2*) has stressed the similarity between the receptors for these drugs and the N-demethylating enzyme present in the liver.

The process of N-demethylation appears to be an enzymatic oxidative reaction resulting in the breaking of a C—N and a C—H bond, as evidenced by formaldehyde formation (*3*). If the rate of demethylation is dependent on the ease with which the C—H bond is oxidized, and if the biological actions are a function of such N-demethylation, then a change in the C—H bonding force would similarly affect both phenomena. To test this hypothesis, morphine in which the N-methyl group has been completely deuterated has been prepared and studied in vivo and in vitro.

To prepare morphine—N—CD_3, normorphine was treated with excess ethyl chloroformate. The resulting N-ethoxycarbonyl group was reduced with lithium aluminum deuteride, giving morphine—N—CD_3, identical to the corresponding protium compound in melting point and ultraviolet absorption spectrum. However, the pK_a of morphine—N—CD_3 was found to be 8.17 as compared to 8.05 for morphine, thus making the deuterium compound a stronger base by 24 percent (*4*).

Alterations in the pharmacological activity of the resulting deuteriomorphine and/or in its oxidative N-demethylation by rat liver microsomal enzymes might suggest a relationship between the methyl group and drug action. Accordingly, the LD_{50}'s for morphine and deuteriomorphine were determined in swiss albino mice by the subcutaneous and intracerebral routes and the ED_{50}'s for analgesia as tested by the tail flick method of D'Amour and Smith (*5*) by the subcutaneous and the intravenous routes. It is apparent from Table 1 that deuteriomorphine is less potent than the parent compound in all categories tested. This is not due to slower absorption of the N—CD_3 compound since it is also less potent than the N—CH_3 compound when administered by the intracerebral and intravenous routes. In addition, passage through the organism does not seem to be slowed, since the onset of action and maximal effect and duration of action of ED_{95} doses was the same for both compounds. Thus, two of the several actions of morphine in mice—death by central nervous system stimulation and analgesia as measured by prolongation of reaction time to a thermal stimulus —are similarly influenced by deuterium substitution in the N—CH_3 group. A change in the potency of sympathomimetic amines upon deuteration in the α-position has recently been reported by Belleau and Burba (*6*).

These workers, however, observed an intensification as well as prolongation of effect on the nictitating membrane. Since the morphine molecule has been changed only by alteration of the