close to the heart of the advance of molecular genetics and epitomizes many issues of the envisioned "biological revolution." Earlier and fuller public analysis of the consequences of nuclear fission, insecticides, fossil fuels, or antibiotics might have moderated or avoided some of today's less desirable consequences.

Is there something to be lost by a "high visibility" assessment? This is again debatable. A Washington-based extravaganza in the polarizing light of the mass media certainly is not needed. The procedures adopted must avoid this. The substantive activity is only in small part suitable for Capitol hearing rooms. In large part it belongs in secluded conference rooms and individual studies. Yet somehow the overall process must be observable and eventually widely shared. Will such visibility elevate public unease to hysteria, thereby cutting off the additional insight that is the only sure antidote to uncertainty? Hopefully not, if the analysis is designed and conducted appropriately. Is this kind of issue better resolved in informed inner circles rather than in the view of a general population that some believe does not have sufficient background for sound judgment? Here the bite of the doctrine of "informed consent" and the weight of "sunshine politics" must take precedence over the nervous concerns of the expert and the professional.

There is, therefore, really no choice but to take this new broader second step, even at the risk of some confusion and inconvenience. Recombinant DNA technology and its associated issues need to be opened to full discussion and the widest understanding under appropriate auspices. If the potential hazards of this step prove manageable, public confidence will be elevated, and the traumas of Hiroshima and environmental pollution may be partly compensated. If the hazards of recombinant DNA technology should prove unmanageable but are concealed, the rate of advance of knowledge may be slowed by far more than inconvenience. If the whole process goes well, science, technology, and the world will each breathe more easily, knowing that on this issue they live openly and honorably, each with the other.

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4. This statement has benefited from the comments and discussion of more than a dozen colleagues in Class II of the National Academy of Sciences. I do not name them to avoid any suggestion of their individual or collective endorsement. Their very different points of view, nonetheless, contributed substantially to the final form of the statement and I am grateful to each of them.

The Histocompatibility System in the Warao Indians of Venezuela

Warao exhibit restricted polymorphism in A, B, and C loci, HLA system, and simplicity of the HLA-D locus.

Zulay Layrisse, Miguel Layrisse, Heinz Dieter Heinen, Johannes Wilbert

The Warao are a tribe of Amerindians who inhabit the Delta region of the Orinoco River in northeastern Venezuela. Several smaller groups of Warao have also settled in adjacent areas to the northwest and the southeast of the Delta proper (60° 40′ to 62° 25′W; 8° 25′ to 10° 5′N). The entire tribe does not exceed 16,000 individuals. With its labyrinthian network of waterways and its islands of dense pluvial forests, the Orinoco Delta has offered refuge to its inhabitants from expanding tribes of Arawakan and Cariban affiliations (*1*).

The tribal name is a self-denomination meaning "Boat People." The Warao are small in stature, measuring 160 cm and less (*1–3*). Travelers through their territory have often commented on the strongly developed thorax and arms and weaker lower extremities of these Indians. That the observed somatological characteristics of the Boat People are the result of environmental adaptation was confirmed by Gardner (*4*), who found that scores for the leg strength of the Warao were significantly below the scores obtained for other Indians.

According to reliable demographic information, the Warao are experiencing a population explosion. The women show a high rate of fertility, averaging 5.4 live births per woman of all ages. The average reaches 8.5 for women with completed reproductive age. The average number of surviving offspring per woman is 3.7, a number that increases for women over 40 years of age to 5.6, or 69 percent of live births (*3*). This figure is very high for tribal populations, where it is not uncommon to find average values below four surviving offspring per woman past reproductive age (*5*).

From the time they were discovered, the Warao have had only sporadic contact with Europeans. Until the 1930's, they did occasionally venture out of the Delta to trade overseas with Trinidad or upstream with Angostura (Ciudad Bolívar). For centuries, however, their basic livelihood depended upon swamp scavenging and riverine and coastal resources, as well as on the systematic exploitation of *Mauritia* sago. It is only recently that horticulture was introduced among them. Nowadays, the Warao plant fields of ocumo, bananas, and maize. Some manioc is also grown and rice serves as a cash crop (*1, 3, 6*).

The Warao are grouped into several independent subtribes. Each subtribe consists of several bands of 25 to 60 individuals that live in one or more villages. The bands of a subtribe form marriage alliances, assist each other in the acquisition of food, and converge for ritual gatherings. Marriage between secondary cousins is preferred but, in the

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absence of an ideal partner, marriage has been observed to occur between first cousins and between uncles and nieces. Generally speaking, marriage is monogamous, but high-ranking elders and some other individuals may have two or more wives; such polygynous marriages are commonly of the sororal type. About 25 percent of the Warao population results from polygynous families. The majority of all marriages are arranged within the subtribe, that is, they take place between members of the related bands of a subtribe. Only in isolated cases does an outsider marry into the subtribe. The mean inbreeding coefficient of all individuals varies between subtribes from 0.01 to 0.04 at the end of five generations. It is very likely, however, that these values are too low (*3*) and probably reflect the fact that the members of the first generation were considered as unrelated, a highly improbable circumstance for this type of society.

Extensive anthropological fieldwork has been conducted among the Warao Indians. These investigations include the study of gene markers in erythrocyte, serum, and leukocyte blood group systems.

Erythrocyte Systems

The distribution of the erythrocyte blood group systems in these Indians shows a lack of the genes A and B of the ABO system and K of the Kell system (*1*). This finding corroborates the historical information, according to which the Warao have largely remained isolated from Creole populations.

The Warao as a whole, but also as individual village populations, exhibit some genetic frequency characteristics that have not been observed either in such neighboring tribes as the Cariña, Pemon, and Acawaio or in more distant tribes as the Macushi, Wapishana, Makiritare (Yekuana), and Yanomami (*1*, *3*). The very high frequency of *N* (.550), especially *NS* (.370), the low frequency of *P* (.150), the high frequency of Fy^a (.870), the low frequency of Jk^a (.250), and the near absence of Di^a (less than .010) are particularly relevant in this respect. Special mention must be made of the frequencies of the *MNS* and Diego systems because they provide particularly useful insights into the dynamics of the dispersive process of gene frequencies in this tribe.

Blood group studies performed in several Warao subtribes have consistently recorded a high frequency of the gene *N* which usually traveled with the gene *S*. Only the population of the Sakobana subtribe was eccentric in this respect; it showed a frequency of .900 for the gene *M* and .830 for the gene *s*. Genealogical analysis of the subtribe produced an explanation for this variation. It was observed that 21 out of 48 individuals representing the second generation came from a single polygynous family in which the father was *MSs* and his three wives *Ms*. This predominance of *M* and *s* in the second generation exercised a distorting influence on the *MNSs* pattern of the subtribe as a whole. At the same time, this particular evidence provides an excellent example of the "founder effect" phenomenon, a variety of genetic drift.

Sakobana was also the only subtribe positive for the Diego gene. The genealogy showed that the gene was carried by members of a certain family which could be traced through three generations. The members of this same family also carried the transferrin D_{chi}, otherwise absent among the Warao tested. Both Di^a and D_{chi} genes do occur in Cariban tribes who live in proximity to the Warao. It is possible, therefore, that the atypical occurrence of these two genes among the Sakobana is the result of their introduction from such populations into the Warao tribe. Another example of this genetic process was recorded by Chagnon *et al.* (*7*). These investigators demonstrated how two Di^a genes of the Diego system and an acid phosphatase type A were introduced into a Yanomami village by members of a particular Makiritare village. Of peculiar interest in this case was the fact that the introduced genes flourished among the Yanomami so vigorously that their frequency rose to become higher than in the Makiritare villages. These findings are examples of a variety of gene drift in small populations, called the "lineal effect," which is a consequence of gene flow into a population by a single individual or a family of different genetic pattern.

The Warao were tested for six serum

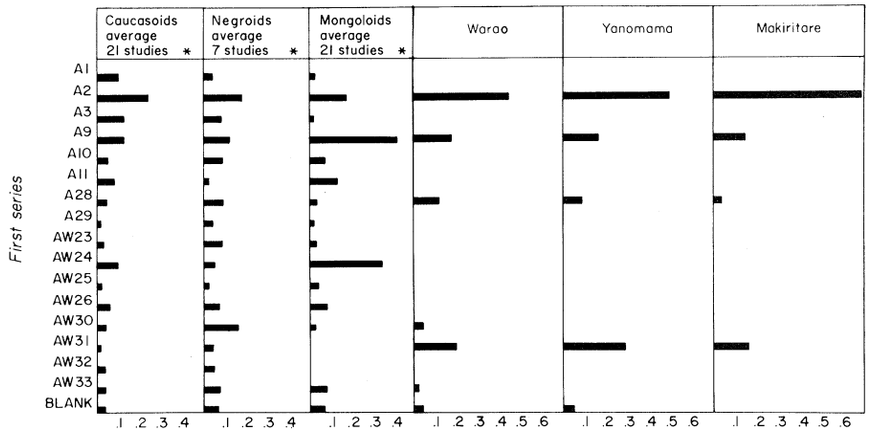

Fig. 1. Gene frequencies of the HLA locus A alleles in the main ethnic stocks of human populations and in Venezuelan Indians. Asterisks indicate average results of studies performed at the 5th International Histocompatibility Workshop.

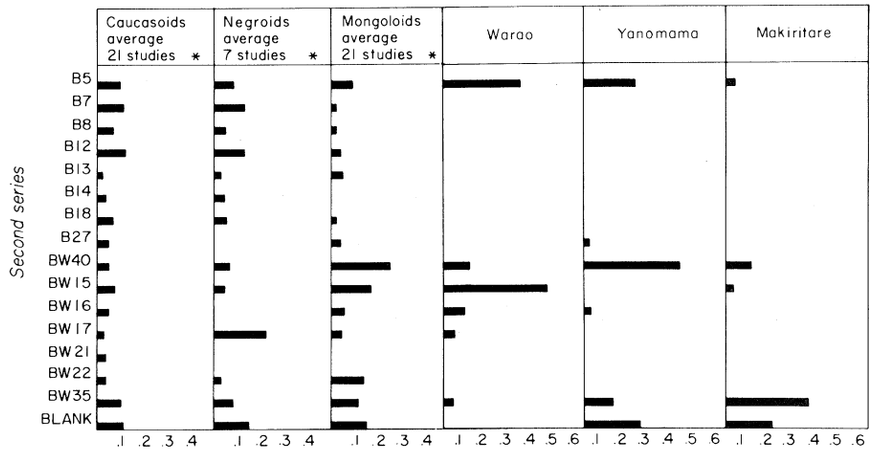

Fig. 2. Gene frequencies of the HLA locus B alleles in the main ethnic stocks of human populations and in Venezuelan Indians. Asterisks indicate average results of studies performed at the 5th International Histocompatibility Workshop.

blood group systems. The results produced no relevant information with the exception of a slow electrophoretic migration of a serum albumin variant observed in a family of the Winikina subtribe (*3*, *8*).

The HLA system

The HLA tissue antigen system is probably the most suitable genetic tool for the analysis of genetic polymorphism in populations and for the determination of the ethnic groups that have substantially contributed to their given pools. Three series of serologically determined alleles constitute the system, with 19 identified antigens in the first series, 20 in the second, and 5 in the third. The combination of these three series brings about 1900 different haplotypes which can serve to identify an ethnic stock as well as a population in which some haplotypes have flourished and become very frequent.

Haplotypes can also be of use for the studies of associations between HLA antigens and diseases. For instance, the antigen B27 shows a significantly higher frequency in *Ankylosing spondylitis* than in the general population, but in this disease, B27 is found more often traveling with A2, AW30, and A9 as compared to all the other A locus alleles. The frequency of B17 is significantly higher in *Psoriasis* and in this condition B17 is usually found traveling with A1 and A2 (*9*).

Much information on the polymorphism of the HLA system resulted from the Fifth International Histocompatibility Workshop when 21 different populations were studied for 16 antigens of the first series and 15 of the second (*10*). The presence of certain genes and the absence of others was observed in populations belonging to the same human division. For example, Mongoloid populations are characterized by a high frequency of genes for A2 and A9 of the first series and high frequencies of genes for BW15, BW22, BW40 of the second (Fig. 1). The genes for AW31 and AW32 of the first and genes for B14 and BW21 of the second series are absent in most of these populations.

The average gene frequencies of Caucasoid populations were found to be very close to those found in Negroid populations. However, the frequencies of genes for A1, B5, B12 and BW35 are usually higher in Caucasoids than in Negroids. Additional useful information is obtained from an examination of individual populations; thus, A3 is in high frequency in Lapps, BW17 and B18 in Sardinians, and A7 and A28 in Tuaregs.

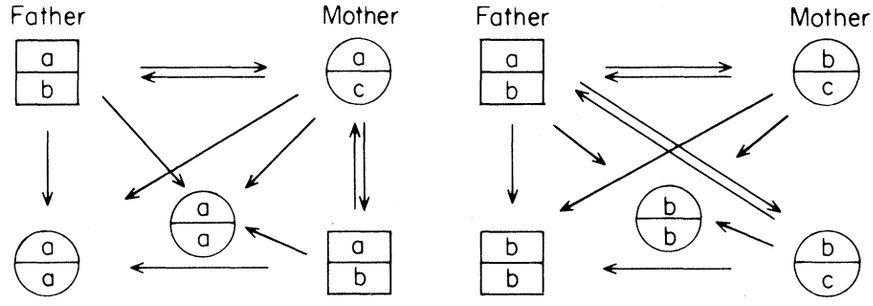

Fig. 3. Mixed lymphocyte cultures between members of two Warao families. Stimulation indexes above three are shown by arrows with heads toward the responders' cells. All possible combinations were tested but negative results are not indicated. Letters are HLA-D determinants assigned according to MLC results.

The HLA gene distribution has been tested in three Venezuelan Indian tribes that show lack of interbreeding with non-Indian populations: the Yanomami and Makiritare (*11*) and the Warao (*12*). These two studies were performed with purified lymphocyte suspensions obtained from heparinized blood and highly selected monospecific antiserums in the cytotoxicity assay of Terasaki and McClelland (*13*). The results indicate a very restricted polymorphism; only six genes of the first series and six genes of the second were detected. As is characteristic for descendants of Mongoloids, these three tribes showed high frequencies of genes for A2 and A9 of the first series. The fact that the gene for AW31 showed a uniformly high frequency among the three tribes is of particular interest because this gene does occur with frequencies below 5 percent in Caucasoids and Negroids but is absent or occurs at very low frequency in Mongoloids (Fig. 2).

Regarding the haplotypes, A1-B8 and A3-B7 are more frequent in Nordic populations and A10-B8 in Far Eastern populations; AW30-B8, AW31-B14, and A9-B14 are common in Negroids from Zambia; A9-B5 in Congolese; and A9-BW17 in Bushmen. The most common haplotypes among the Warao are A2-B5, A2-BW15, and AW31-BW15. The common ones among the Yanomami are AW31-BW40, A2-B5, and A2-BW40. It is interesting that the high frequency of BW15 among the Warao and its absence among the Yanomami parallels the absence and high frequency, respectively, of asymptomatic Australia antigen (Au) carriers in these tribes (*14*), confirming thus the observation made among Australian aborigines of deficiency of BW15 among Au carriers (*15*).

Seven other American Indian tribes were examined by the participants of the aforementioned international workshop. In general, the native populations showed similarly high frequencies of the same genes observed among the tested Venezuelan tribes. They also carried several other genes that most likely originated from non-Indian admixture.

The third HLA series has only recently been worked out; only five genes have been identified and many remain to be detected, as indicated by the high proportion of "blanks." In all populations tested the most frequent alleles among the Caucasoids are CW3 and CW4, with values ranging from .100 to 0.201 and .077 to 0.125, respectively. Among the African blacks the most frequent alleles are CW2 (.101) and CW4 (.098) and among the Japanese, CW1 and CW3 with frequencies of .017 and .024, respectively. Of particular interest is the very low frequency of CW1 (.002) in African blacks and of CW2 ($< .001$), CW4 (.002), and CW5 ($< .001$) in Japanese (*16*). Among the Warao only CW1 and CW3 have been observed, with frequencies of .130 and .400, respectively, and the well-known association between CW3 and BW15 observed consistently in all populations was also present among this tribe (*17*).

Stimulation in mixed lymphocyte culture (MLC) is governed by alleles of a locus within the main histocompatibility complex. This histocompatibility system, previously known as LD-1 or MLC-1, is now included in the HLA system as its fourth or D series. Identification of the specificities in the mixed lymphocyte culture response has been possible through the use of cells from LD homozygous offspring of haplo-identical matings in inbred and outbred populations. At the last international histocompatibility workshop eight "clusters" of closely associated determinants were identified and it is postulated that several additional ones will soon become incorporated into this system (*18*).

The Warao population is highly inbred and shows a striking homogeneity of HLA determinants. The overall frequency of homozygous individuals, calculated from only two HLA loci, gave a value of .0936 for Winikina village, which is almost five times the frequency of homozygotes of the HLA system calculated by Bodmer for Caucasoids and African populations (*19*). Consequently, these Warao Indians are a potential source of LD homozygous subjects. A study of the MLC response was conducted in 75 related and unrelated individuals living in several villages of the Winikina subtribe (*20*). Unidirectional MLC tests were set up with lymphocyte suspensions purified on a Ficoll-Hypaque gradient; 1.5×10^5 responding and 3×10^5 stimulating cells (irradiated with ^{60}Co) were prepared in a final volume of 0.2 ml (*21*). Cultures were kept at 37°C for a total of 144 hours, 1 μc of [^{3}H]thymidine being added during the last 16 hours. Stimulation indexes were obtained by dividing the radioactivity (counts per minute) of the allogeneic mixture (AB_x) by the radioactivity of the responder's autostimulated control mixture (AA_x). Values above three were considered as indicating the occurrence of stimulation. Positive MLC reactions were observed in only one out of 32 HLA-identical sibling pairs. Combinations of lymphocytes from closely related kin such as parent-child, grandparent-grandchild, uncle-nephew, and first cousins showed stimulation in about 17 percent of the combinations. There was a further increase of stimulation (up to 39 percent of the combinations) when pairs of HLA identical unrelated individuals were tested. The values obtained for the Warao rate far below those reported for panmictic populations in which the stimulation occurs in 90 percent of HLA identical unrelated individuals.

Further studies of the Winikina subtribe of the Warao demonstrated the existence of several individuals homozygous for LD determinants (*22*). They were identified by MLC's performed within members of selected families; Fig. 3 illustrates two of the families, where a pair of siblings behave in MLC as homozygous for different LD determinants. During the Workshop one of these homozygous Warao samples (a/a) identified group LD 108 of the LD system (*18*). Other homozygous samples suggested the possibility of another determinant (b/b) present also in high frequency among the Warao. Studies are currently under way to determine whether LD 108 is a common gene in Indian populations.

The sophisticated techniques for the detection of blood systems such as HLA impose a heavy burden on the team of investigators. Expeditions to Indian territory are complex in logistic procedure beyond the normal measure; sample collection, transportation, and uninterrupted laboratory work must take place at an accelerated speed. While methods of preservation of the various constituents of blood have improved a great deal in recent years, they cannot be fully employed in field situations. New methods are needed to facilitate the work in hinterlands. Within the next decade many of the remaining Indian populations will become absorbed in the Creole population, and in the process their social structures and cultures will disintegrate. We are faced with our last opportunity to study an important aspect of the evolution of mankind.

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