INDIVIDUAL BLOOD DIFFERENCES IN MEXICAN INDIANS, WITH SPECIAL REFERENCE TO THE Rh BLOOD TYPES AND Hr FACTOR*

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Recent investigations have shown that there are striking differences in the distribution of the Rh blood types in different races (1). To date, studies have been carried out on white individuals (2, 3), Negroes (4), Chinese (5), Japanese (6, 7), and Asiatic Indians (8). Because the results already obtained have proved to be of value for the study of racial relationships, it seems worth while to carry such studies further.

In this paper, we propose to present the results of Rh typings on a series of 98 Mexican Indians and to compare the distribution of the Rh blood types in Mexican Indians with that of other races. The blood samples of these Mexican Indians have also been tested for the A-B-O groups, the subgroups of A, the M-N types, the agglutinogen P, and the Hr factor. While a number of studies have already been carried out on the blood groups, subgroups of A and M-N types in American Indians (9–11), and while there has also been one study on the Rh factor (12), thus far no data have been collected for this race concerning the Rh blood types, or the properties P and Hr.

Materials and Methods

The Mexican Indians studied by us came from Tuxpan, this locality being selected because of the special assistance[1] available there and because the Indians there kept themselves apart from the Mexicans and whites.

In prehistoric times, Tuxpan is said to have been inhabited by nomad tribes of unknown origin. The existence of these tribes is inferred from the primitive art work they left behind, in particular very crude pottery and rudimentary stone carving. The Indians themselves are descendants of the family of Toltecas, which migrated from a certain locality known as Tollan (also called Huehuetlapallan) in the United States. In their trek from the United States to the state of Jalisco, the Toltecas Indians passed through Sinaloa, stopped near the city of Tepic for a short time, and then moved on to Tuxpan which they reached in the year 542 A.D., according to Fr. Antonio DeTello. The bulk of the tribe then moved on to the valley of Mexico, but a nucleus of the tribe remained behind in Tuxpan. These may have mixed with the nomad tribes and were the ancestors of the Indians tested by us. During the Spanish

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domination of Mexico, the only white people who came in contact with the Indians at Tuxpan were the government employees but they did not intermarry with the Indians. White people came to Tuxpan about 1812. According to the statistics available, 90 per cent of the present population of Tuxpan are full-blooded Indians. Tuxpan is located at 19° 22′ north latitude and 4° west longitude from the meridian of Mexico City. It is 1,137 meters above sea level and its climate is temperate. It is a fertile valley surrounded by mountains and there are two volcanoes in the locality.

The blood samples were collected by venipuncture by one of us (J. P. Z.) from 100 Indians at Tuxpan, Mexico, and shipped at once by air express to New York City where they arrived within 7 days. The blood samples were received on a Sunday morning and no tests were made until the following day. It took 3 days for all the tests to be completed. At the beginning of each day's work blood suspensions were prepared by shaking up the clots in saline solution; coarse particles were allowed to settle and the supernatant smooth suspensions transferred to a series of numbered tubes. The suspensions were washed once by centrifuging them at low speed and resuspending the sediment in fresh saline solution, to form a 2 per cent suspension (in terms of blood sediment). At the end of each day's experiments all the blood cell suspensions were discarded and fresh suspensions were prepared the following morning from the blood clots which were kept in the refrigerator during the interim.

As in our previous studies, the anti-A and anti-B sera used were of high titer and avidity, having been prepared from the sera of donors who had been injected intravenously with plasma (Sharpe and Dohme), or intramuscularly with solutions of A and B group substances.[2] The absorbed B serum was prepared from the blood of a group B individual whose serum contained an unusually large amount of a_1 agglutinin. The anti-M and anti-N testing fluids were prepared in the usual manner by absorption of our stock rabbit immune sera.

For Rh typing, sera of specificities anti-Rh_0 (standard, 85 per cent positive for bloods of white individuals), anti-Rh′ (70 per cent positive), and anti-Rh″ (30 per cent positive) are required. The anti-Rh_0 serum used in the present study came from a group B, type Rh′ mother of an erythroblastotic infant of type Rh_1. The serum was of high titer (128) and for the tests was used in a dilution of 1:8, by mixing 1 part of serum, 1 part of autoclaved group A saliva solution (or group substance), and 6 parts of saline. Two different reagents of specificity anti-Rh′ were used: one from an Rh-negative mother of an erythroblastotic infant, the second from a type Rh_2 mother of an erythroblastotic infant. The serum from the former individual was of specificity anti-Rh_0' (87 per cent positive) with weak anti-Rh_0 agglutinins. The anti-Rh′ reagent was prepared from this serum by blocking the action of the anti-Rh_0 agglutinin by using the following formula: 1 part of the stock serum was mixed with 1 part each of potent blocking serum and pooled A and B saliva solution and further diluted with 2 parts of saline solution. The second anti-Rh′ serum, from the type Rh_2 mother, besides being of high titer, had the advantage that it contained the anti-Rh′ agglutinin in pure form without any anti-Rh_0 agglutinin. For this reagent the following formula gave excellent results: 1 part stock serum was mixed with 1 part pooled A and B saliva solution and 3 parts of saline solution. Two anti-Rh″ reagents were also used, one prepared with the aid of blocking serum from an anti-Rh_0'' serum and the second from the serum of a type Rh_1 mother of an erythroblastotic infant. The two anti-Rh″ reagents were prepared from these two stock sera in a manner similar to that used for the anti-Rh′ reagents.

The anti-Hr serum was exceptionally potent and was made available to us through the courtesy of Dr. Peter Vogel. It came from a group O, type Rh_1 mother of an erythroblastotic infant. Its exceptional potency may be evident from the fact that all Hr-positive bloods gave uniform strong reactions. The single gene and double gene dose effect first noticed by Race

[2] For this material we are indebted to Dr. J. A. Leighty of the Eli Lilly Company.

and Taylor (13), and also observed by Wiener, Davidsohn, and Potter (14) was not evident with this serum because this effect is only apparent with weaker anti-Hr sera. Despite its exceptionally high titer, the anti-Hr serum was used undiluted for testing the samples of all the group O Indians. For testing Indians belonging to groups A and B, it was first mixed with an equal volume of pooled A and B saliva.

The anti-P serum came from a group A individual who had become isoimmunized to the agglutinogen P as a result of repeated blood transfusions (15). This serum was diluted with an equal volume of saline solution for the tests, and was not used for testing the few group B blood samples in the series.

With regard to the saliva solution used for the inhibition of the anti-A and anti-B agglutinins in the anti-Rh sera, we have found the following to be the simplest and most satisfactory method of preparing it. Equal volumes of group A and B saliva from secretors were pooled and autoclaved at 15 pounds pressure for 15 minutes. The coagulated mucus and other suspended material were then readily removed by centrifugation. The supernatant, opalescent fluid was diluted with 2 volumes of saline solution and distributed among a number of vials of convenient size. After a second autoclaving for 15 minutes at 15 pounds pressure, the vials were capped with sterile rubber stoppers and stored in the refrigerator until used. If the saliva could not be processed immediately after collection, it was placed in a boiling water bath for 15 minutes to destroy the blood group enzymes and then stored in the refrigerator until processing could be carried out.

The A-B and M-N tests were carried out on Boerner well slides at room temperature as described in our previous papers. All group A bloods were further tested with absorbed B serum. The Rh typings and Hr tests were carried out in small test tubes in a water bath at 38° C. as already described (2, 14). The tests for agglutinogen P were also carried out in test tubes but at refrigerator temperature (15).

Of the 100 blood samples received, two were hemolyzed and therefore not tested because it was felt the results would be unreliable. The findings on the remaining 98 blood samples are presented in Table I.

Blood Groups, Subgroups, and M-N Types

In Table II, we have summarized the results of the tests for the blood groups, subgroups of A, and M-N types on the 98 Mexican Indians. For the purpose of comparison, we have also listed in this table the groups, subgroups of A, and M-N types of a different group of Indians and of white individuals in New York City.

The Indians in both studies differ from whites in the high incidence of group O. At one time it was held that all full-blooded Indians belonged to group O (16), but Matson and Schrader (17) encountered a number of Indian tribes with a high frequency of group A (as high as 76.7 per cent). The two Indian tribes in Table II show somewhat different distributions of the blood groups and they both contain significant numbers of group A individuals. All of the group A individuals in the Indian tribes listed in Table II belong to subgroup A_1, in contrast to the situation in white individuals where as many as one-fourth or one-fifth of all A or AB individuals belong to subgroup A_2. These results are in agreement with those of Levine, Matson, and Schrader (18) who

report that subgroup A_2 is virtually absent even in Indian tribes with a high frequency of group A. In this connection, it should be mentioned that sub-

TABLE I

Classification of Blood Samples from 98 Mexican Indians

1. $OMNRh_2Hr^+P^+$	26. $BMNRh_1Rh_2$	51. $OMRh_1Rh_2Hr^+P^+$	76. $OMRh_1Hr^-P^-$
2. $OMRh_1Rh_2Hr^+P^+$	27. $OMRh_2Hr^+P^+$	52. $OMRh_1Rh_2Hr^+P^+$	77. $OMRh_1Rh_2Hr^+P^+$
3. $OMNRh_2Hr^+P^+$	28. $ONRh_1Hr^-P^+$	53. $OMRh_1Hr^-P^-$	78. $OMNRh_1Rh_2Hr^+P^+$
4. $OMRh_1Rh_2Hr^+P^+$	29.* $OMNRh_1Hr^-P^+$	54. $OMRh_1Hr^+P^+$	79. $OMNRh_1Hr^-P^+$
5. $OMRh_1Hr^-P^+$	30. $OMNRh_2Hr^+P^+$	55. $OMRh_1Rh_2Hr^+P^+$	80. $OMRh_2Hr^+P^-$
6.* $OMNRh_1Rh_2Hr^+P^+$	31. $OMRh_1Hr^-P^+$	56. $OMRh_1Rh_2Hr^+P^+$	81. $OMNRh_1Rh_2Hr^+P^+$
7. $OMNRh_1Hr^-$	32. $OMRh_1Hr^-P^+$	57. $ONRh_1Hr^-P^+$	82. $OMNRh_1Hr^-P^+$
8. $OMRh_1Rh_2Hr^+P^+$	33. $OMRh_1Hr^-P^+$	58. $OMRh_1Hr^-P^+$	83. $OMNRh_1Hr^-P^-$
9. $OMRh_1Rh_2Hr^-P^-$	34. $OMRh_2Hr^+P^+$	59. $OMRh_1Rh_2Hr^+P^+$	84. $OMNRh_1Hr^-P^+$
10. $OMRh_1Rh_2Hr^+P^+$	35. $OMNRh_1Rh_2Hr^+P^+$	60. $OMRh_1Rh_2Hr^+P^+$	85. $OMRh_1Rh_2Hr^+P^+$
11. $OMRh_1Rh_2Hr^+P^+$	36. $OMNRh_1Hr^-P^+$	61 $OMRh_1Hr^-P^-$	86. $OMRh_1Rh_2Hr^+P^+$
12. $A_1MNRh_1Rh_2Hr^+P^+$	37. $OMRh_1Hr^-P^+$	62. $OMRh_1Hr^+P^+$	87. $A_1MRh_1Hr^+P^+$
13. $OMRh_1Hr^-P^-$	38. $OMRh_1Hr^-P^+$	63. $OMRh_1Rh_2Hr^-P^+$	88. $OMRh_2Hr^+$
14. $OMRh_1Rh_2Hr^-P^+$	39. $OMNRh_1Rh_2Hr^+P^+$	64. $OMNRh_1Hr^-P^+$	89. $OMRh_1Hr^+P^+$
15. $BMNRh_2Hr^+$	40. $OMRh_1Hr^-P^+$	65. $OMRh_1Hr^-P^+$	90. $OMRh_1Rh_2Hr^+P^-$
16. $OMNRh_1Hr^-P^+$	41. $OMNRh_1Rh_2Hr^+P^+$	66. $OMNRh_1Rh_2Hr^+P^+$	91. $OMRh_1Hr^-P^+$
17. $OMNRh_1Hr^-P^+$	42. $OMNRh_1Rh_2Hr^+P^-$	67. $OMRh_1Rh_2Hr^+P^+$	92. $OMRh_1Hr^-P^+$
18. $A_1NRh_1Hr^-P^+$	43. $OMNRh_1Hr^+P^+$	68. $OMRh_1Rh_2Hr^+P^+$	93. $OMNRh_1Rh_2Hr^+P^+$
19. $OMRh_1Hr^-P^-$	44. $A_1MRh_1Rh_2Hr^+P^+$	69. $OMNRh_2Hr^+P^-$	94. $OMRh_1Rh_2Hr^+P^-$
20. $A_1MRh_1Hr^-P^+$	45. $OMRh_1Hr^+P^-$	70. $OMNRh_1Hr^-P^-$	95. $OMRh_1Rh_2Hr^+P^-$
21. $A_1MNRh_1P^-$	46. $OMRh_1Hr^-P^+$	71.* $BMNRh_1Rh_2Hr^+$	96. $OMRh_1Hr^-P^+$
22. $OMNRh_1Rh_2Hr^+P^+$	47.* $OMRh_0Hr^+P^+$	72. $OMRh_1Hr^-P^+$	97. $OMNRh_1Hr^-P^+$
23. $OMRh_1Hr^+P^+$	48. $OMRh_1Hr^-P^+$	73. $OMNRh_1Rh_2Hr^+P^-$	98. $OMNRh_1Rh_2Hr^+P^+$
24. $OMRh_1Rh_2$	49. $OMRh_1Rh_2Hr^+P^+$	74. $OMNRh_1Rh_2Hr^+P^+$	
25. $OMRh_1Hr^-P^-$	50. $OMRh_1Hr^-P^+$	75. $OMRh_1Hr^-P^-$	

* Indians known to be mixed.

TABLE II

Distribution of the Agglutinogens A_1-A_2-B and M-N among 98 Mexican Indians As Compared with U. S. Indians and Whites

Population	Investigators	No. of individuals tested	Distribution of groups and subgroups						Distribution of types		
			0	A_1	A_2	B	A_1B	A_2B	M	N	MN
			per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Mexican Indians	Present study	98	90.8	6.1	0	3.1	0	0	61.2	3.1	35.7
Full-blooded Indians (various tribes) Fort Lewis, Wash.	Landsteiner, Wiener, and Matson (12)	120	73.3	25.8	0	0.8	0	0	56.7	4.2	39.2
Whites (U. S.)	Wiener (10)	1,077	41.7	29.0	8.9	13.9	5.2	1.4	29.2	21.3	49.5

group A_2 is lacking or extremely rare in Chinese (5) and Japanese (7), an observation which supports the theory that American Indians are of Mongolian origin.

It is not certain whether the group B individuals among the Mexican Indians we tested derived their B gene from their Mongolian ancestors or from mixture

with other races. While Snyder (16) has demonstrated that the presence of individuals of groups A, B, and AB in many Indian tribes is due to mixture with whites or other races, it would be impossible to explain on this basis frequencies of group A as high as 75 per cent, as has been reported by Matson and Schrader for Blackfeet Indians. Moreover, Boyd (19) has proved statistically that even in Indian tribes with low frequencies of groups A and B, the distributions are usually inconsistent with the theory of racial admixture. A simple way of testing for admixture is to subgroup the group A individuals, as was done in the present study; the presence of individuals of subgroup A_2 would indicate the presence of racial admixture. As shown in Table I, with only four exceptions, all the Indians tested in the present study are believed to be full-blooded.

It will be seen from Table II that the distribution of the M-N types in the two Indian tribes is very similar, and strikingly different from the distribution in whites. It is of interest to mention that all the Indian tribes tested to date

TABLE III

Distribution of Agglutinogen P among Mexican Indians As Compared with Other Races

Population	Investigators	No. of individuals tested	Frequencies of types		Frequencies of genes	
			P+	P−	*P*	*p*
			per cent	*per cent*	*per cent*	*per cent*
Mexican Indians.....	Present study	95	78.9	21.1	54.1	45.9
Whites (N. Y. C.).....	Wiener and Unger (15)	328	73.2	26.8	48.2	51.8
Negroes (N. Y. C.).....	Wiener and Unger (15)	73	97.7	2.3	84.9	5.1

(10), including the original study by Landsteiner and Levine (11), also exhibit very similar distributions for the M-N types. The question may arise how it is possible for tribes of American Indians with such widely different distributions of the blood groups to resemble one another so closely with respect to the M-N types. A possible explanation is that the blood groups are determined by a series of three allelic genes, while the M-N types are determined by a pair of allelic genes, so there is greater leeway for variation in the former case.

The Agglutinogen P

The observations on agglutinogen P may be of interest because so few studies have been carried out thus far on this property. In the original study of Landsteiner and Levine (20), these investigators pointed out the striking difference in the distributions of agglutinogen P among whites and Negroes. Aside from the studies of Dahr and his collaborators (21) on white individuals in Germany, and the studies by Furuhata and his collaborators (22) on the related property Q in Japanese, the only other studies on the agglutinogen P are those listed in Table III. Although the number of Mexican Indians tested is small, it will

be seen that the distribution of property P is significantly different from that in Negroes, there being a substantial number of P-negative individuals, just as in whites.

The Rh Blood Types

The results of the tests for the Rh blood types on the 98 Mexican Indians are summarized in Table IV. Inasmuch as the Rh blood types were only recently discovered, it seemed of interest, for purposes of comparison, to summarize in the table all the available data on the distribution of these types in different races.

TABLE IV

Distribution of the Rh Blood Types among Mexican Indians As Compared with Other Races

Population	Investigators	No. of individuals tested	Distribution of Rh blood types								Gene frequencies					
			Rh⁻	Rh_1	Rh_2	Rh_1Rh_2	Rh_0	Rh′	Rh″	Rh′Rh″	*rh*	Rh_1	Rh_2	Rh_0	*Rh′*	*Rh″*
			per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Mexican Indians	Present study	98	0	48.0	9.2	41.8	1.0	0	0	0	0	68.1	30.0	1.9	0	0
Whites (N. Y. C.)	Wiener, Unger, and Sonn (3)	1,468	13.3	54.4	13.7	15.0	2.4	1.0	0.2	0	36.5	43.3	14.5	3.2	1.3	0.3
Negroes (N. Y. C.)	Wiener, Belkin, and Sonn (4)	223	8.1	20.2	22.4	5.4	41.2	2.7	0	0	28.4	11.7	14.4	42.1	2.7	0
Asiatic Indians	Wiener, Sonn, and Belkin (8)	156	7.1	70.5	5.1	12.8	1.9	2.6	0	0	26.6	56.2	6.0	3.4	4.4	0
Chinese	Wiener, Sonn, and Yi (5)	132	1.5	60.6	3.0	34.1	0.8	0	0	0	1.9	77.0	20.7	0.4	0	0
Japanese	Waller and Levine (6)	150	1.3	37.4	13.3	47.3	0	0	0	0.7	0.4	61.8	37.8	0	0	0
Japanese	Miller and Taguchi (7)	180	0.6	51.7	8.3	39.4	0	0	0	0	2.1	70.2	27.7	0	0	0

As shown in the table, the distribution of the Rh blood types among the Mexican Indians differs strikingly from the distribution in whites, Negroes, and Asiatic Indians. The absence of Rh-negative individuals conforms with the previous report of Landsteiner, Wiener, and Matson (12),[3] who tested a series of North American Indians using anti-*rhesus* guinea pig serum. As in the Chinese and Japanese, the Mexican Indians belong almost exclusively to types Rh_1, Rh_2, and Rh_1Rh_2, and no bloods giving intermediate reactions (23) were encountered. These findings, therefore, further support the theory of Mongolian derivation of the American Indians.

The calculation of the gene frequencies is simplified if one makes use of the

[3] At the time this study was made the eight Rh blood types were not known. However, some of the Indians in the study were tested with anti-Rh′ sera.

fact that the eight Rh blood types fall into four natural pairs, giving rise to the four classes as follows:

$$W = Rh_0 + \text{Rh negative} \tag{1}$$

$$U = Rh_1 + Rh' \tag{2}$$

$$V = Rh_2 + Rh'' \tag{3}$$

$$UV = Rh_1Rh_2 + Rh'Rh'' \tag{4}$$

As was pointed out in previous papers, these four classes are transmitted by triple allelic genes just like the four blood groups. Accordingly, the frequencies of the three genes can be calculated with the aid of the following formulae:

$$w = \sqrt{W} \tag{5}$$

$$u = \sqrt{W + U} - \sqrt{W} \tag{6}$$

$$v = \sqrt{W + V} - \sqrt{W} \tag{7}$$

If one wishes to compute the frequencies of the six Rh genes, the following formulae are first used:

$$rh = \sqrt{\text{Rh neg.}} \tag{8}$$

$$Rh' = \sqrt{Rh' + \text{Rh neg.}} - \sqrt{\text{Rh neg.}} \tag{9}$$

$$Rh'' = \sqrt{Rh'' + \text{Rh neg.}} - \sqrt{\text{Rh neg.}} \tag{10}$$

The frequencies of the other genes are then obtained by subtraction, using the following relationships:

$$w = Rh_0 + rh \tag{11}$$

$$u = Rh_1 + Rh' \tag{12}$$

$$v = Rh_2 + Rh'' \tag{13}$$

While these formulae are not 100 per cent efficient, they are consistent and were satisfactory for calculating the gene frequencies for the populations of whites, Negroes, and Asiatic Indians listed in Table IV. The formulae become highly inefficient, however, whenever the frequency of any of the three classes W, U, V is very low. The formulae were therefore unsatisfactory for the Mexican Indians, Chinese, and Japanese because of the low frequency of class W. Accordingly, the following formulae were used instead for these races.

$$u = 1 - \sqrt{(Rh'^-)} \tag{14}$$

$$v = 1 - \sqrt{(Rh''^-)} \tag{15}$$

$$w = 1 - (u + v) \tag{16}$$

where (Rh′-) represents the percentage of individuals whose blood gave negative reactions with the anti-Rh′ serum, and (Rh″-) represents the percentage of negative reactions with the anti-Rh″ serum.

Inasmuch as there were no Mexican Indians of types Rh′, Rh″, Rh′Rh″, or Rh negative, the frequencies of genes *Rh′*, *Rh″*, and *rh* were all zero or very small. Therefore, for this race:

$$Rh_1 = u \tag{17}$$

$$Rh_2 = v \tag{18}$$

$$Rh_0 = w \tag{19}$$

It is of interest to apply the chi square test to our sample of Mexican Indians. This is done by recalculating the four classes with the aid of the formulae:

$$W = w^2 \tag{20}$$

$$U = u^2 + 2uw \tag{21}$$

$$V = v^2 + 2vw \tag{22}$$

$$UV = 2uv \tag{23}$$

Accordingly, the expected frequencies of the four classes are as follows:

$$W = Rh_0 = (0.019)^2 = 0.04 \text{ per cent, or } 0.04 \text{ individuals} \tag{24}$$

$$U = Rh_1 = (0.681)^2 + 2(0.681)\,(0.019) = 49.0 \text{ per cent, or } 48 \text{ individuals.} \tag{25}$$

$$V = Rh_2 = (0.30)^2 + 2(0.30)\,(0.019) = 10.1 \text{ per cent, or } 9.9 \text{ individuals.} \tag{26}$$

$$UV = Rh_1Rh_2 = 2(0.681)\,(0.30) = 40.9 \text{ per cent, or } 40.1 \text{ individuals.} \tag{27}$$

The observed frequencies of the Rh types were:

$$Rh_0 = 1$$
$$Rh_1 = 47$$
$$Rh_2 = 9$$
$$Rh_1Rh_2 = 41$$

Therefore,

$$\chi^2 = \Sigma \frac{(x - x_0)^2}{x_0} = \frac{(1 - 0.04)^2}{0.04} + \frac{(47 - 48)^2}{48} + \frac{(9 - 9.9)^2}{9.9} + \frac{(41 - 40.1)^2}{40.1}$$

$$= 23.0 + 0.2 + 0.08 + 0.02 = 23.1$$

Since n = 1, P is much less than 0.01.

These findings, therefore, do not conform with the expectations under the theory. The difficulty obviously centers about the single individual encountered of type Rh_0. This might be attributed to chance because only a single individual is involved, except that the same difficulty has arisen in the studies on Chinese and Japanese. This suggests some complication in the genetic

mechanism. The nature of this complication will be discussed in connection with the Hr factor.

The Hr Factor

In Table V are listed the results of the Hr tests on 95 of the Mexican Indians, and, for comparison, also a series of white individuals and a series of Negroes. It will be seen that the incidence of Hr-negative individuals is highest among the Mexican Indians and lowest among the Negroes.

According to the genetic theory of Race *et al.* (24), the Hr factor is present in the agglutinogens determined by genes Rh_2, Rh'', Rh_0, and rh but not in the agglutinogens determined by genes Rh_1 and Rh'. The frequency of the Hr-negative type should therefore be equal to the square of the sum of the frequencies of genes Rh_1 and Rh'. As shown in Table V, the observed frequencies

TABLE V

Distribution of the Hr Factor among Mexican Indians As Compared with Other Races

Population	Investigators	No. of individuals tested	Frequencies of types		$(Rh_1 + Rh')^2$
			Hr+	Hr−	
			per cent	*per cent*	*per cent*
Mexican Indians....	Present study	95	55.8	44.2	46.4
Whites.....	Wiener, Davidsohn, and Potter (14)	239	72.0	28.0	19.9
Negroes.....	Wiener, Davidsohn, and Potter (14)	49	98.0	2.0	2.1

agree closely with the expected values for the Mexican Indians and Negroes, but the frequency of the Hr-negative type among white individuals is too high, suggesting some complication in the genetic mechanism.

Among the Mexican Indians tested by us, presumably only three of the six allelic Rh genes are present, namely, Rh_1, Rh_2, and Rh_0 (*cf.* Table V). Under these conditions, the Rh types which are possible, the corresponding genotypes, the Hr reactions to be expected, and the calculated frequencies of the phenotypes are as follows:—

Rh types	Genotypes	Hr reactions	Frequencies
Rh_0	Rh_0Rh_0	Positive	w^2
Rh_1	Rh_1Rh_1	Negative	u^2
	Rh_1Rh_0	Positive	$2uw$
Rh_2	Rh_2Rh_2, Rh_2Rh_0	Positive	$v^2 + 2vw$
Rh_1Rh_2	Rh_1Rh_2	Positive	$2uv$

It will be seen that Hr-negative individuals are theoretically possible only in type Rh_1. Moreover, using the calculated frequencies given in equation

(25) the expected proportion of Hr-negative individuals in type Rh_1 is $\frac{u^2}{u^2 + 2uw} = \frac{0.464}{0.490}$ or 95 per cent. That is, only 1 out of 20 Mexican Indians belonging to type Rh_1 would be expected to be Hr-negative, and the observed number (7) of such individuals in our series of 98 Indians does not differ significantly from the expectations (*cf.* Table VI).

TABLE VI

Relation of the Hr Factor to the Rh Blood Types among 91 Mexican Indians

Hr reaction	No. of individuals in types				Totals
	Rh_0	Rh_1	Rh_2	Rh_1Rh_2	
Hr^+	1	7	9	36	53
Hr^-	0	39	0	3	42
Totals	1	46	9	39	95

TABLE VII

Relation of the Rare Genes Rh_y and Rh_z to the Six Standard Rh Genes

Genes	Reactions with Rh antisera			Reaction with Hr antiserum
	Rh′	Rh″	Rh_0	
rh	Neg.	Neg.	Neg.	Pos.
Rh_0	Neg.	Neg.	Pos.	Pos.
Rh′	Pos.	Neg.	Neg.	Neg.
Rh_1	Pos.	Neg.	Pos.	Neg.
Rh″	Neg.	Pos.	Neg.	Pos.
Rh_2	Neg.	Pos.	Pos.	Pos.
Rh′ ″ (Rh_y)	Pos.	Pos.	Neg.	Neg.
$Rh_{1\ 2}$ (Rh_z)	Pos.	Pos.	Pos.	Neg.

In Table VI is given a comparison of the Hr reactions and the Rh blood types in the 95 Mexican Indians. It will be seen that, contrary to expectations, as many as 3 out of the 39 Mexican Indians belonging to type Rh_1Rh_2 were Hr-negative. This observation seemed so startling to us that the tests were repeated a number of times, but always with the same results. Race *et al.* (24) have already encountered a few Hr-negative individuals of type Rh_1Rh_2 among Caucasians and on this basis postulated the existence of one or possibly two rare genes (Rh_y and Rh_z) in addition to the six standard Rh genes (25). The reactions determined by the rare genes Rh_y and Rh_z in comparison with the six standard genes are given in Table VII. As indicated in the table, these two

genes are perhaps more appropriately named *Rh′ ″* and $Rh_{1\ 2}$, respectively, in keeping with the designations for the other Rh genes (26, 27). According to some unpublished observations of Wiener, Sonn, and Unger, the genes *Rh′ ″* and $Rh_{1\ 2}$, appear to be quite rare among white individuals, because only one Hr-negative individual was found among 132 individuals of type Rh_1Rh_2 in contrast to the relatively high incidence of this phenotype among Mexican Indians.

Since the effects of genes *Rh′ ″* and $Rh_{1\ 2}$ would be practically indistinguishable among Mexican Indians because they would occur in combination with Rh_1, or Rh_2 or Rh_0, in the succeeding discussion, for the sake of simplicity, we shall consider only gene $Rh_{1\ 2}$. If we assign to this gene the frequency x, the Rh types, their theoretically corresponding genotypes, the Hr reactions to be expected, and the theoretically expected frequencies of the six possible phenotypes would be as follows:—

Rh types	Genotypes	Hr reactions	Theoretical frequencies	Observed frequencies: Absolute	Observed frequencies: Per cent
Rh_0	Rh_0Rh_0	Positive	w^2	1	1.1
Rh_1	Rh_1Rh_1	Negative	u^2	39	41.1
	Rh_1Rh_0	Positive	$2uw$	7	7.4
Rh_2	Rh_2Rh_2, Rh_2Rh_0	Positive	$v^2 + 2vw$	9	9.5
Rh_1Rh_2	Rh_1Rh_2, $Rh_{1\ 2}Rh_2$, $Rh_{1\ 2}Rh_0$	Positive	$2uv + 2vx + 2wx$	36	37.9
	$Rh_{1\ 2}Rh_1$, $Rh_{1\ 2}Rh_{1\ 2}$	Negative	$2ux + x^2$	3	3.1

The theory can be tested by computing the values of the four gene frequencies w, u, v, and x, and using these values to recalculate the frequencies of the six phenotypes in order to apply the chi square test.

Since,

$$u^2 = 0.411$$

Then,

$$u = \sqrt{0.411} = 0.641 \text{ or } 64.1 \text{ per cent}$$

Moreover,

$$2uw = 0.074$$

So that,

$$2(0.641)\, w = 0.074$$
$$w = 0.058 \text{ or } 5.8 \text{ per cent}$$
$$Rh_0 + Rh_2 = w^2 + 2vw + v^2 = 0.106$$
$$w + v = \sqrt{0.106} = 0.326 \text{ or } 32.6 \text{ per cent}$$

Therefore,

$$v = 26.8 \text{ per cent}$$
$$\text{and } x = 1 - (u + v + w) = 1 - 0.967 = 0.033 \text{ or } 3.3 \text{ per cent}$$

Using these values for the four gene frequencies, the expected absolute frequencies of the six phenotypes are as follows: $Rh_0 = 0.3$; Rh_1 (Hr^-) = 39.0; Rh_1 (Hr^+) = 7.0; $Rh_2 = 9.8$; Rh_1Rh_2 (Hr^+) = 34.7; and Rh_1Rh_2 (Hr^-) = 4.1.

Therefore,

$$\chi^2 = \frac{(1 - 0.3)^2}{0.3} + 0 + 0 + \frac{(9 - 9.8)^2}{9.8} + \frac{(36 - 34.7)^2}{34.7} + \frac{(3 - 4.1)^2}{4.1}$$

$$= 2.04, \text{ for } 1^\circ \text{ of freedom}$$

So that, P = 0.16

Accordingly, the agreement between the observed frequencies and those expected under the genetic theory is satisfactory.

In conclusion, it should be mentioned that in view of the similarity in distribution of the Rh blood types among Chinese, Japanese, and American Indians, it would be reasonable to expect a similar incidence of the genes *Rh′ ″* and/or $Rh_{1\ 2}$ in these peoples.

SUMMARY

98 Mexican Indians were tested for the blood properties A-B-O, A_1-A_2, M-N, P, Rh′-Rh″-Rh_0-rh, and Hr. Of the 98 Indians, 90.8 per cent belonged to group O, 6.1 per cent belonged to A_1, and 3.1 per cent to group B. There were 61.2 per cent of type M, 3.1 per cent of type N, and 35.7 per cent of type MN. Of the 95 Mexican Indians tested with anti-P serum, 21.1 per cent were found to lack the P agglutinogen.

In tests for the Rh blood types, 48.0 per cent of the Indians were found to belong to type Rh_1, 9.2 per cent to type Rh_2, 41.8 per cent to type Rh_1Rh_2, and 1 per cent to type Rh_0. There were no bloods giving intermediate reactions. Of the 95 Indians tested for the Hr factor 44.2 per cent were found to lack this property.

The reactions for the Rh blood types and Hr factor were correlated with each other and the results supported the conclusion of Race *et al.* that in addition to the six standard allelic genes and the so called intermediate genes, there is one or possibly two genes having the property of determining agglutinogens which react with anti-Rh′ and anti-Rh″ sera, but not with anti-Hr serum. This gene (or genes) appears to be relatively common among Mexican Indians (approximately 3.3 per cent) in contrast to its rareness in white individuals.

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