Studies on the "Group Specific Component" of Human Serum. Gene Frequencies in Several Populations *

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IN 1959 HIRSCHFELD DESCRIBED several variants of a normal serum protein (Hirschfeld, 1959b). The variants are distinguishable by immunoelectrophoresis (Grabar and Williams, 1953) as indicated by their different mobilities on agar gel. The protein belongs to the α-globulin fraction. It is easily distinguishable from the haptoglobins and so far not identified with one of the already isolated and characterized proteins of this fraction (Cleve and Bearn, 1961). Hirschfeld and co-workers, who have arbitrarily called this protein the "Group Specific Component", have presented evidence that the variants are under genetical control (Hirschfeld, Jonsson, and Rasmuson, 1960).

The material presented in this paper includes:

1. Studies on families, twin-pairs, and mother-child pairs to investigate further the mode of inheritance.

2. Results of gene frequency determinations in an American white population, American and African Negroes, North American Indians (Navajos), Eskimos, and an American Chinese population.

METHODS

Immunoelectrophoresis: In this study the microtechnique of Scheidegger modified by Hirschfeld was used (Scheidegger, 1955; Hirschfeld, 1959a). This modification consists essentially of a longer separation time and the use of a barbital buffer to which calcium lactate has been added (Laurell, Laurell, and Skoog, 1956). These modifications result in a better resolution of the overcrowded α-globulin region.

Electrophoresis was carried out at pH 8.6 for 100 minutes employing a potential gradient of about 7 volts/cm. The antiserum used was the horse-antiserum against pooled normal human serum obtained from the Commercial Service of the Pasteur Institute, Paris, France (Sérum équin anti-sérum humain normal, No. 13411 and 13412, Serpasteur, Paris).

MATERIALS

Families: The sera of 31 families with 64 offspring from a U. S. white population were studied. The material included apparently healthy families, and

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families from patients with Wilson's disease, agammaglobulinemia, or chromosomal aberrations.

Sera from 14 families of Northern Nigeria (Habe) were obtained. Studies on the haptoglobin and transferrin inheritance in this population have been published by Barnicot, Garlick, and Roberts (1960). The material included 25 matings with 60 offspring. Two matings, found to be inconsistent in ABO transmission and inheritance of other blood group factors (Barnicot, Garlick, and Roberts, 1960), also revealed inconsistencies in transmission of Gc and were excluded.

Sera from 11 families with 43 offspring from the Navajo Indians were available. Sera of 34 twin-pairs comprising 17 monozygotic and 17 dizygotic, and 25 samples composed of serum from mothers and the cord-blood of their new born infants were studied.

Populations: 1. U. S. white population: Sera from 122 unrelated individuals collected in New York City from blood donors and patients of The Rockefeller Institute Hospital. 2. American Negro population: Sera from 144 unrelated individuals collected in New York City from blood donors and patients of The Rockefeller Institute Hospital. 3. U. S. Chinese population: Sera from 117 unrelated individuals collected in New York City from blood donors and patients of general practitioners. These individuals were of maternal and paternal Chinese ancestry; their origin could be traced to the Kwangtung province in southern China. 4. Eskimos: Sera from 67 unrelated individuals collected by The Hamilton Health Association, Hamilton, Ontario, Canada. The Eskimos were residents from different parts of Northern Canada. Sixty-two individuals were residents from Baffin Island, four from Northern Quebec and one from Prince of Wales Island. 5 and 6. Northern Nigerians (Hausa): The material has been described in a publication on the haptoglobin and transferrin inheritance in Norther Nigerians (Barnicot, Garlick, and Roberts, 1960). The samples were collected by Dr. D. F. Roberts, Dept. of Anatomy, University of Oxford, England from fifty-two villages within a radius of 40 miles south of Katsuma, Northern Nigeria. They were obtained from two ethnic groups, Fulani and Habe. Fulani sera from 100 unrelated individuals were collected at random. Habe: 54 samples of unrelated individuals were collected at random and 49 unrelated individuals were obtained from the family material. 7. Navajo Indians: The samples were collected on the reservation of Manyfarms, Arizona by the Department of Public Health, Cornell University Medical College, New York, N. Y. The material included 152 unrelated individuals and 86 individuals from families of various sizes. The families consist of either one parent and children or sibs without available parents. The offspring of matings, where both parents were recorded, have been rejected.

THE THREE PHENOTYPES OF THE GROUP SPECIFIC COMPONENT

The immunoelectrophoretic analysis of different normal human sera is illustrated in Fig. 1. The antiserum used has revealed 18 precipitin lines, indicating 18 different antigenic components. In the α-globulin fraction differences in the electrophoretic position of precipitation lines were observed. The precipitation

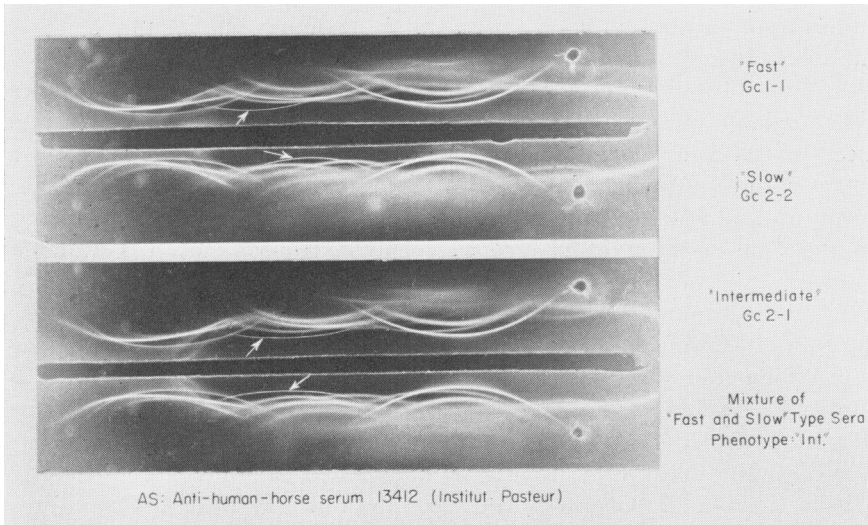

FIG. 1. A comparison of the immunoelectrophoretic patterns of the three Gc phenotypes. A comparative immunoelectrophoretic pattern of a mixture of the fast and slow phenotypes is also illustrated. The Gc is indicated by arrows. The separation has been carried out from right (cathode) to left (anode).

line of an α-globulin with three different relative electrophoretic mobilities was delineated. A fast moving component was observed in some sera (Fig. 1, first sample). In others the precipitate had the position of a slow moving component (Fig. 1, second sample). The third group of sera showed a long, rather flattened precipitation line (Fig. 1, third sample). Under optimal conditions this precipitate had two peaks, the position of the peaks corresponded to the precipitates of the fast and slow moving components. All normal human sera examined could be classified into one of these three groups. The three phenotypes may be called "Fast", "Intermediate" (or "two-peaked") and "Slow". Reproducible and clearcut variations of the "Fast" and "Intermediate" phenotypes have been observed in our laboratory and their genetical significance is under study.

The reproducibility of the classification of serum types has been investigated. Serum tested repeatedly over a nine month period and several samples of serum from the same individual, stored at $-15°C$ over a two year period, showed no alterations in phenotypes. Sera that were contaminated or stored at room temperature for a long period of time showed alterations of the immunoelectrophoretic pattern of several serum proteins. In particular, alteration of electrophoretic mobilities or the shapes of the precipitates occurred. Correct typing of the Gc groups was not possible in these instances.

STUDIES ON THE GENETIC MECHANISM

The results of the determination of the group specific component in 67 families with 167 offspring are summarized in table 1.

The family data suggest that the Gc is controlled by two co-dominant auto-

TABLE 1. GC-PHENOTYPES OF PARENTS AND CHILDREN

Parental Phenotypes	No. of Familes	Offspring			Total
		Fast	Int.	Slow	
U.S. White					
Fast-Fast	7	9	—	—	9
Fast-Int.	16	21	20	—	41
Fast-Slow	4	—	6	—	6
Int.-Int.	3	3	3	1	7
Int.-Slow	1	—	1	—	1
Slow-Slow	0	—	—	—	0
	31	33	30	1	64
Nigerian					
Fast-Fast	14	29	—	—	29
Fast-Int.	10	17	11	—	28
Int.-Int.	1	—	2	1	3
	25	46	13	1	60
Navajo					
Fast-Fast	11	43	—	—	43

somal alleles. In a fashion analogous to the notation of the haptoglobin groups, the notation Gc 1-1, Gc 2-2, and Gc 2-1 has been suggested for the serum types "Fast", "Slow", and "Intermediate", and Gc^1 and Gc^2 for the genes (Hirschfeld and Beckman, 1960).

Thirty-four twin pairs were studied; in 17 monozygotic twins the group specific component was concordant; in 17 dizygotic twins, 13 pairs had concordant types, and 4 pairs had discordant types.

Comparison of maternal and cord sera was made in 25 mother-child pairs. In 18 cases concordant Gc types were observed, in 7 cases the Gc types were discordant. In 5 cases the mother was heterozygous and the child homozygous for the Gc types in two cases the mother was homozygous and the child heterozygous. In addition to the confirmation of the suggested genetical mechanism these results indicate that the presence of the group specific component in sera of newborns is not due to placental transfer of the protein, a possibility considered because previous investigations from this laboratory had indicated that the group specific substance has a relatively slow s-rate of approximately 4S. The discordance of genotypes in mother-child pairs indicate that the fetus synthesizes its own group specific component.

The immunoelectrophoretic analysis of a mixture of equal amounts of sera from the two homozygotes reveals the phenotype characteristic of the heterozygote (Fig. 1, fourth sample).

GENE FREQUENCIES IN SEVERAL POPULATIONS

The distribution of Gc genotypes in seven populations is given in table 2. Striking differences were observed. Gene frequencies were calculated by maxi-

TABLE 2. GC-GENOTYPES IN VARIOUS POPULATIONS

Population	Total	Gc 1-1		Gc 2-1		Gc 2-2	
		No.	%	No.	%	No.	%
Eskimo	67	34	50.75	26	38.80	7	10.45
U. S. White	122	63	51.64	49	40.16	10	8.20
U. S. Chinese	117	72	61.54	36	30.77	9	7.69
U. S. Negro	144	116	80.56	25	17.36	3	2.08
Nigerian Habe	103	88	85.44	15	14.56	—	0
Nigerian Fulani	100	90	90.00	10	10.00	—	0
Navajo	245	235	95.92	9	3.67	1	0.41

TABLE 3. ESTIMATION OF GENE RATIO FOR GC-GENES IN A NAVAJO INDIAN POPULATION

Number in Family*	Total Indiv.	a Gc 1-1	b Gc 2-1	c Gc 2-2	$b + 2a = x$	$2(a + b + c) = y$	Weight per Gene w
Unrelated	159	152	7	—	311	318	1.000,000
2	46	43	2	1	88	92	.666,667
3	15	15	—	—	30	30	.500,000
4	4	4	—	—	8	8	.400,000
5	15	15	—	—	30	30	.333,333
6	6	6	—	—	12	12	.285,714
	245	235	9	1	479	490	

S (wx) = 401.296 $Gc^1 = 0.9765$
S (wy) = 410.962 $Gc^2 = 0.0235$

* Family consists of either one parent and children or children alone.

mum likelihood estimates, and in the Navajo population by Cotterman's gene weighting method (Cotterman, 1947). Application of the gene weighting method to the Navajo population is demonstrated in table 3. Gene frequencies for Gc^1 and Gc^2 and standard errors (s′) of the estimates are given in table 4. The frequency for Gc^2 varies from 0.30 in Eskimos to 0.02 in Navajo Indians. Eskimos, whites, and Chinese have relative high frequencies of Gc^2, 0.30, 0.28, and 0.23, respectively. Gc^2 is lower in frequency in American Negroes (0.11). The two populations of African Negroes studied have relatively low frequencies of Gc^2, 0.07 (Habe) and 0.05 (Fulani). Gc^2 is rare in Navajo Indians, where the frequency was 0.02.

Homogeneity-χ^2-tests indicate significant differences between the gene frequencies of the white, American Negro, African Negro, and Navajo populations. The gene frequencies among the Eskimos, whites, and Chinese were not significantly different. Similarly the two populations from Northern Nigeria did not differ significantly. The frequencies in the U. S. white population differ slightly from that in a Swedish population, where the frequency of Gc^2 was 0.26 (Hirschfeld, Jonsson, and Rasmuson, 1960).

Goodness of fit of the observed and expected distribution according to the Hardy-Weinberg equilibrium has been calculated by Chi-square (table 5). Because of the relatively small size of the samples and the rareness of Gc^2 in the

TABLE 4. FREQUENCIES OF Gc ALLELES IN VARIOUS POPULATIONS

Population	Gc^1	Gc^2	s′
Eskimo	0.7015	0.2985	0.0395
U. S. White	0.7172	0.2828	0.0289
U. S. Chinese	0.7693	0.2307	0.0275
U. S. Negro	0.8924	0.1076	0.0183
Nigerian Habe	0.9272	0.0728	0.0181
Nigerian Fulani	0.9500	0.0500	0.0154
Navajo	0.9765	0.0235	0.0075

s′ = standard error of the gene frequency estimates

TABLE 5. GOODNESS OF FIT OF HARDY-WEINBERG EQUILIBRIUM IN VARIOUS POPULATIONS

Population	χ^2	P
Eskimo	0.361	>.50
U. S. White	0.012	>.90
U. S. Chinese	2.080	>.10
U. S. Negro	1.335	>.20
Nigerian Habe	0.635	>.30
Nigerian Fulani	0.277	>.50
Navajo	8.577	>.01

two Northern Nigerian populations and the Navajo Indian population the formula developed by Levene has been applied for the calculation of the expected number of the various genotypes (Levene, 1949). The Chi-square tests for goodness of fit differ only very slightly from the figures given in table 5. In the Habe, the χ^2 was 0.587, $P > .30$; in the Fulani 0.252 and $P > .50$. In the Navajo Indians after application of Cotterman's gene weighting method and using Levene's formula a χ^2 value of 3.386 was found, $P > .05$. All the populations are in equilibrium except the Navajo Indians where the very low frequency of Gc^2 does not permit meaningful equilibrium calculations.

The observed differences in gene frequencies indicate that the group specific component will be a valuable additional marker for the characterization of populations and potentially useful in calculations on the dynamics of hybrid populations (Glass and Li, 1953; Steinberg, Stauffer, and Boyer, 1960). A reasonable calculation of the admixture of genes from white ancestors in the American Negro population should await the collection of more extensive data on gene frequencies in African Negroes.

SUMMARY

1. The three phenotypes of the group specific component of human serum have been described. The mode of inheritance as a two allelic system without dominance suggested by Hirschfeld and co-workers, was confirmed.

2. Gene frequencies in Eskimos, U. S. whites, U. S. Chinese, American Negroes, African Negroes, and Navajo Indians were reported. The frequency of Gc^2 was relatively high in Eskimos, U. S. whites, and U. S. Chinese, and was lower in American and African Negroes. Gc^2 was relatively rare in Navajo Indians.

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