Two Genetic Variants of the Group-Specific Component of Human Serum: Gc Chippewa and Gc Aborigine

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THE GROUP-SPECIFIC COMPONENTS (Gc) are serum α_2-globulins which comprise a human serum protein polymorphism distinguishable by immunoelectrophoresis (Hirschfeld, 1959). Variations in relative electrophoretic mobility permit the classification of individual sera into three common phenotypes. Some sera contain a fast migrating component (Gc 1-1), others a slow migrating component (Gc 2-2), while the third phenotype is characterized by the presence of both components in approximately equal amounts (Gc 2-1). The common phenotypes are controlled by two autosomal co-dominant alleles, Gc^1 and Gc^2, (Hirschfeld, Jonsson and Rasmuson, 1960; Cleve and Bearn, 1961; Reinskou and Mohr, 1962).

In the course of studies on the distribution of the *Gc* alleles in various populations, a number of unusual Gc phenotypes have been observed (Hirschfeld, 1962a, b; Cleve and Bearn, 1962). One phenotypic variation has occurred among Caucasians and consists of a variant which migrates slightly more rapidly than Gc 2-2 and thus lies intermediate between Gc 1-1 and Gc 2-2. This variant has been termed Gc X (Hirschfeld, 1962a). Another variation has been found in the serum of an African Negro. In this instance, the variant migrates slightly more rapidly than Gc 1-1, and has been called Gc Y (Hirschfeld, 1962a). Similar and possibly corresponding variants have been observed in our laboratory in one Caucasian subject and three American Negroes. Further identification of these variants using the additional technique of starch gel electrophoresis has been described in the preceding communication (Parker, Cleve and Bearn, 1963). It has not yet been possible to collect sufficient family material to test the hypothesis that these two rare phenotypic variations are under genetic control, and further investigations are in progress.

In the present report, two new genetically determined variants of the Gc-system will be described. The first was observed in a sample of Chippewa Indians during a study of *Gc* gene frequencies in North American Indians. The second variant was disclosed in a sample collected in the Cape York area of northeastern Australia during the course of an extensive survey of the Australian Aborigine population. These two variants are designated, therefore, as Gc

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Chippewa and Gc Aborigine, respectively. Both variants were originally detected by immunoelectrophoresis, but the recognition of certain phenotypes involving the variant Gc Chippewa was made possible only by application of the modified starch gel procedure. This investigation includes the results of family studies and the determination of the gene frequency of the two alleles in the populations where they were first observed.

METHODS

Immunoelectrophoretic analysis: Sera were examined according to Scheidegger's micromethod as modified by Hirschfeld (Scheidegger, 1955; Hirschfeld, 1959a). A voltage gradient of 6-7 volts/cm was applied for 120 minutes. The antisera used in this study included horse antisera No. 13411 and No. 306 from the Institut Pasteur, Paris, and several rabbit antisera prepared in our laboratory.

The validation of suspected variants of the Gc types by immunoelectrophoresis requires the examination of mixtures of the proband serum with standard sera of known Gc type. The mixtures were obtained by inserting into the origin 1 μl of the proband's serum followed by 1 μl of the standard serum.

Starch gel electrophoresis: The vertical starch gel system of Smithies was utilized with modifications to improve resolution of the Gc bands, as described by Parker, Cleve and Bearn (1963).

CHARACTERIZATION OF THE NEW GENETIC VARIANT Gc CHIPPEWA

Material

The Chippewa Indians are members of the Algonquian linguistic group of North American Indians. Originally, they lived as nomadic hunters and fishers in the Great Lakes region on the northern shores of Lake Huron and in the area surrounding Lake Superior. In the United States today they live on several reservations scattered throughout the northern part of Minnesota, where many are still engaged in their traditional pursuits of fishing and hunting.

The specimens were collected on the Red Lake Reservation, Red Lake, Minnesota. Blood was drawn under sterile conditions into evacuated glass containers (Vacutainer) and sent to New York City. The material arrived in the laboratory within 3 days. In the original survey, 159 individuals were sampled, including some related persons and persons with varying degrees of non-Indian ancestry. For gene frequency analysis, all individuals with recorded non-Indian ancestry were excluded; the remaining sample of full blood Chippewa Indians consisted of 62 individuals. From four individuals of the original sample in whom an unusual Gc phenotype was observed, it was possible to secure a second blood specimen. These four individuals were members of a single kindred. Specimens from 36 additional members of this kindred were obtained and more detailed genealogical information was obtained.

Results

One hundred fifty-nine sera of Chippewa Indians were examined by immunoelectrophoresis and the distribution of the Gc phenotypes was determined. Ninety-four sera were provisionally classified as Gc 1-1, fifty-nine sera as Gc 2-1, and six sera as Gc 2-2. The frequency of Gc^2 was calculated to be 0.223.

It was noticed, however, that several sera provisionally classified as Gc 1-1 and Gc 2-1 revealed an unusual immunoelectrophoretic pattern. In five of 59 sera classified as Gc 2-1, the pattern was clearly distinguishable from the usual heterozygous pattern. As illustrated in Fig. 1, the Gc precipitate in these sera showed a more distinct separation between the peaks of the fast and slow-migrating components than in the usual heterozygous Gc type. The fast component in such sera migrated slightly more rapidly than Gc 1, thereby causing an extension of the precipitate towards the anode. This phenotypic variation was technically reproducible. In some sera, provisionally classified as Gc 1-1, it was noticed that the Gc precipitate seemed to be slightly more extended towards the anode than commonly observed in Gc 1-1 sera, (Fig. 1), but the deviation was small and a clear cut distinction from the common Gc 1-1 type was not obtained.

A modification of the vertical starch gel electrophoresis technique of Smithies (Parker, Cleve and Bearn, 1963) has provided an additional method for the determination of Gc types in human sera. The group-specific components migrate on starch gel in the post-albumin region. The product of the Gc^1 allele is present in the first prominent post-albumin band; the product of the Gc^2 allele is present in the second. Additional post-albumin bands are not related to the Gc-system. Application of this technique to the investigation of the variant types observed in Chippewa Indians permitted a further characterization. The starch gel electrophoretic pattern of the variant types is illustrated in Fig. 2. A prominent band located between the cathodal edge of the albumin band and the Gc^1 band was a characteristic finding in each variant. In the sera originally classified as unusual Gc 1-1 types this distinct band was present together with a prominent band in the Gc^1 position; in the sera originally classified as unusual Gc 2-1 types the band in the fast migrating position appeared together with a prom-

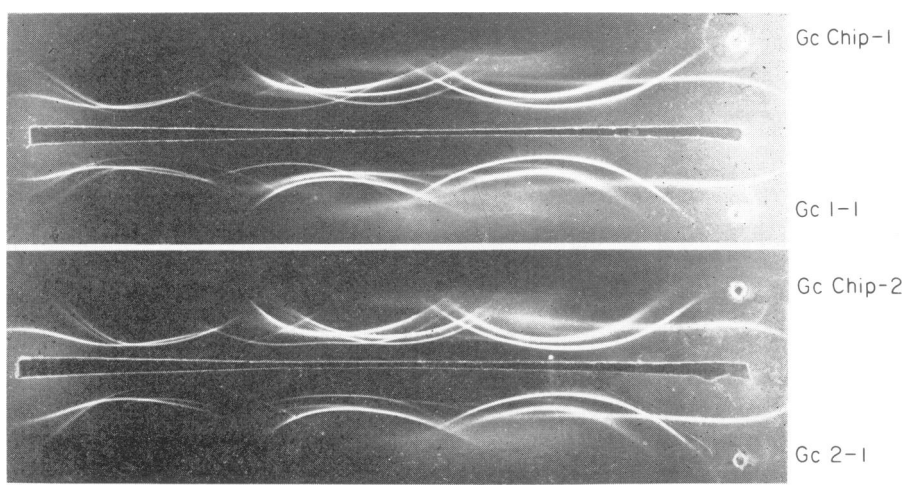

FIG. 1. Immunoelectrophoretic analysis of sera from Chippewa Indians. The phenotype Gc Chippewa-1 is compared with the standard Gc 1-1, and the phenotype Gc Chippewa-2 is compared with the standard Gc 2-1. Note the differences in shape and position of the Gc precipitates between the usual and the variant phenotypes.

inent band in the Gc^2 position. The phenotypic appearance of the variant types on immuno- and starch gel electrophoresis indicates the presence of a faster migrating variant of the group-specific component, which is designated Gc Chippewa. The two unusual Gc types were classified Gc Chippewa-1 and Gc Chippewa- 2 (Gc Chip-1; Gc Chip-2). In sera of the common Gc types, a weak post-albumin component can be seen in a position corresponding to the prominent Gc Chippewa band. From four individuals typed as Gc Chippewa-2, a second sample of blood was obtained after an interval of three months; the phenotypic variation was found to be reproducible. Because of the difficulty in distinguishing between Gc Chip-1 and Gc 1-1 by immunoelectrophoresis, all sera provisionally classified as Gc 1-1 by immunoelectrophoresis were re-examined by starch gel electrophoresis. Of 38 such sera, 10 were classified as Gc Chip-1 and 28 as Gc 1-1.

The genetic control of the Chippewa variant was tested by determination of Gc types in the sera of 41 members of three generations of a large Chippewa kindred (Fig. 3). The kindred contains several informative matings. In four families both parents and a total of 14 offspring were investigated; in four other families, with a total of 14 offspring examined, only one parent was available in each family. In addition, the kindred contains two mother-child combinations. The variant Gc Chippewa was inherited in a pattern consistent with an autosomal, co-dominant allele $Gc^{Chippewa}$ (Gc^{Chip}) at the Gc locus; no exceptions to simple mendelian inheritance were found.

The distribution of the Gc alleles was studied in the sample of 62 fullblood

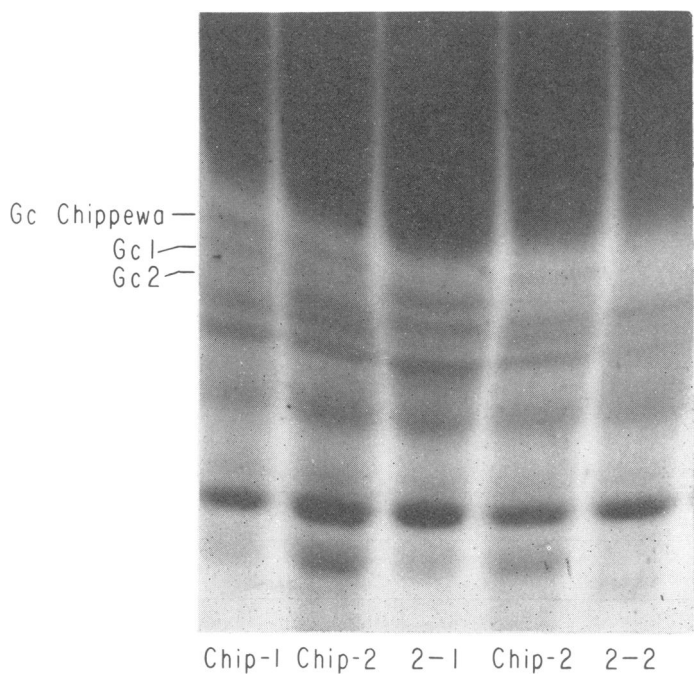

FIG. 2. Demonstration of Gc Chippewa phenotypes by starch gel electrophoresis.

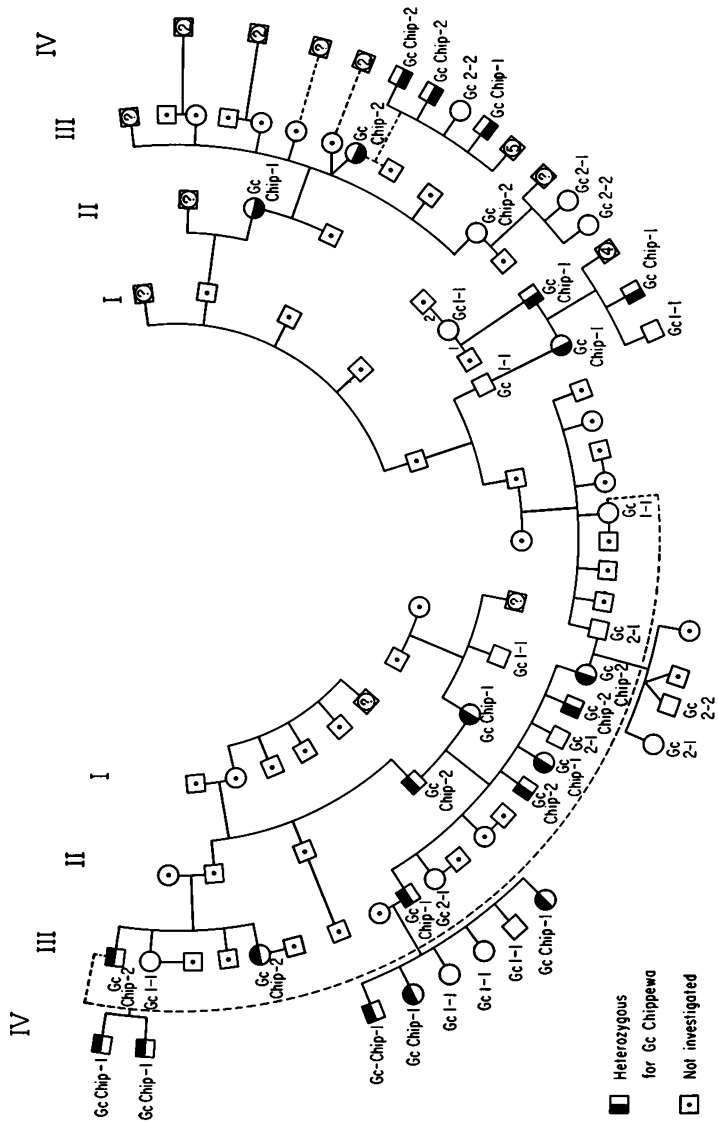

FIG. 3. Pedigree of a kindred of Chippewa Indians illustrating the inheritance of Gc Chippewa.

TABLE 1. DISTRIBUTION OF Gc ALLELES IN 62 CHIPPEWA INDIANS

	Total	Gc Chip-Chip	Gc Chip-1	Gc Chip-2	Gc 1-1	Gc 2-1	Gc 2-2
Obs.	62	0	10	3	28	19	2
Exp.	61.99	0.68	8.92	2.73	20.09	17.84	2.73

Gene Frequencies:
$Gc^{Chip} = 0.105$
$Gc^{1} = 0.685$
$Gc^{2} = 0.210$
χ^2 corrected (Yates) $= 0.1605$
$P > .50$

Chippewa Indians; the results are summarized in table 1. The frequencies of the common alleles were calculated to be 0.685 for Gc^1 and 0.210 for Gc^2. The frequency of the variant allele $Gc^{Chippewa}$ was 0.105. The observed distribution of phenotypes in this small sample was in good agreement with the expected distribution on the basis of the Hardy-Weinberg equilibrium.

CHARACTERIZATION OF THE GENETIC VARIANT Gc ABORIGINE

Material

During an extensive survey of the distribution of Gc alleles in Australian Aborigines, unusual Gc phenotypes were observed in samples collected at the Aurukun Presbyterian Mission. The Aurukun Mission is situated near the mouth of the Archer River on the west coast of the Cape York Peninsula. The Mission is in contact with several hundred Aborigines. The majority of these individuals belong to a group of tribes whose names begin with Wik, particularly the Wik-Mungken, Wik-Eppa, Wik-Ngartona and Wik-Ngencherra. Intermarriage between these groups has been frequent in recent years and at present the entire group is considered as a single unit.

Blood of 74 full blood Aborigines was collected, sent by air to Perth, Western Australia, and the serum stored at -20°C. Aliquots of these samples were sent in dry ice to New York City. It was possible to obtain a second sample from two of the individuals who showed unusual phenotypic variations.

Results

Sera from the 74 individuals were examined by immunoelectrophoresis. Careful analysis of the Gc precipitates in these sera revealed six different Gc phenotypes (Fig. 4) of which three were previously unrecognized. The three unusual Gc phenotypes differ from the common types in possessing a more rapidly migrating variant of the group-specific component called Gc Aborigine. It became apparent that this variant is controlled by an additional allele at the *Gc* locus, $Gc^{Aborigine}$ (Gc^{Ab}). The homozygous type Gc Aborigine-Aborigine (Gc Ab-Ab) contains a group-specific component migrating distinctly faster than Gc 1-1. The precipitate is located in the α_1-globulin region and is arc-shaped in a fashion similar to the precipitates of the homozygous types Gc 1-1 and 2-2. The sera of the heterozygous type Gc Aborigine-1 (Gc Ab-1) show a flatter, extended precipitate covering the area of the faster migrating component and Gc 1-1. The sera of the type Gc Aborigine-2 (Gc Ab-2) contain a precipitate representing the faster migrating component and the gene product of Gc^2. The precipitate is extended from the α_1-globulins to the slow α_2-globulins and reveals two distinctly separated peaks. The position of the anodal peak corresponds to the maximum of the faster migrating component, Gc Aborigine; the position of the cathodal peak corresponds to the location of Gc 2-2. The phenotypic variations were technically reproducible and were verified by comparative analysis with sera of standard Gc types. Additional confirmation was obtained by the investigation of mixtures of sera with variant types and

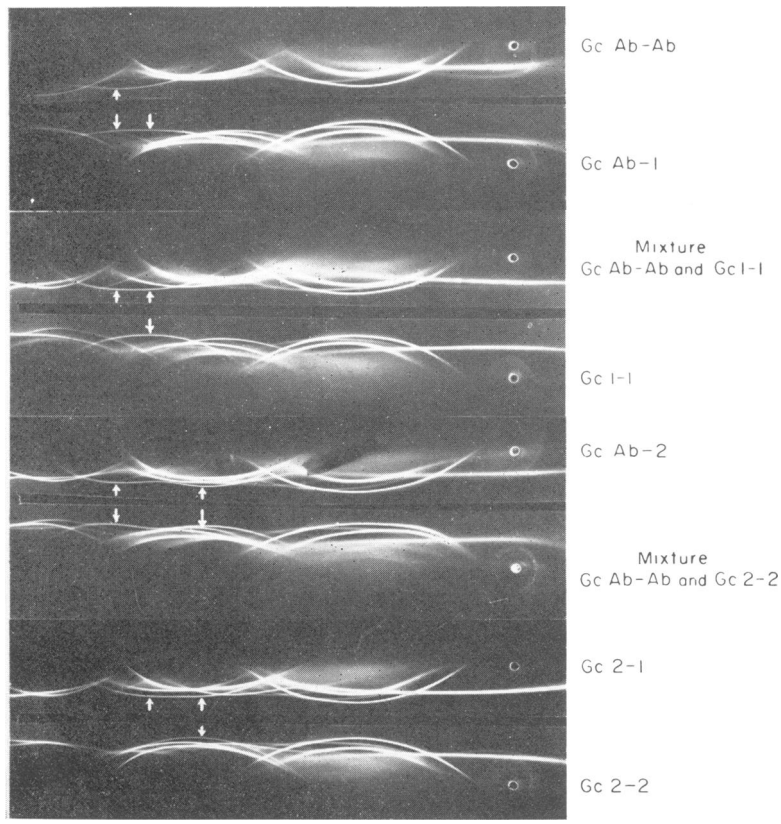

FIG. 4. Immunoelectrophoretic demonstration of the genetic variant Gc Aborigine. The figure shows the common Gc types 1-1, 2-1 and 2-2 and the variant phenotypes Ab-Ab, Ab-1 and Ab-2. The results of mixing the homozygous variant type with homozygous standard sera are also illustrated.

sera with common Gc types. Sera with suspected variant phenotypes were classified only after repeated examination by comparative analysis and by investigation of artificial mixtures. Mixtures of Gc Ab-Ab serum with standard sera of type Gc 1-1 and type Gc 2-2 are also illustrated in Fig. 4. The former reveals a Gc phenotype equivalent to the heterozygous type Gc Ab-1; the latter has the phenotype of the heterozygous type Gc Ab-2.

Comparative immunoelectrophoretic analysis of the variants Gc Chippewa and Gc Aborigine revealed minor differences in relative mobilities. By prolonged electrophoresis (120-140 minutes) Gc Aborigine was observed to migrate slightly more rapidly than Gc Chippewa. By starch gel electrophoresis, a clear difference between the Chippewa and Aborigine variants was disclosed. As previously described, Gc Chippewa migrates between Gc 1 and albumin. A protein band corresponding to Gc Aborigine could not be detected by inspection of the protein-stained starch gel. For the localization of Gc Aborigine, a series of punches (diameter 0.35 cm) extending from the region of the albumin to ceruloplasmin was taken from the bottom slice of the unstained gel. The punches

were re-examined by immunoelectrophoresis on agar gel and Gc Aborigine was localized in the punches taken from the cathodal edge of the albumin band. It may therefore be concluded that using the electrophoretic conditions employed in this experiment, Gc Aborigine migrates within the albumin region.

To test the proposed genetic hypothesis, only a single informative family was available (Fig. 5). Both parents were heterozygous for Gc Aborigine. Among the eight children of this mating all of the possible Gc phenotypes were observed (Gc Ab-Ab, Gc Ab-1, Gc Ab-2, Gc 2-1). It was possible to secure a second sample of serum from the father (Gc Ab-1) and a homozygous daughter (Gc Ab-Ab) after an interval of 15 months. In both sera the demonstration of the phenotypic variation was reproducible and the original classification was confirmed.

The distribution of Gc phenotypes in the sample of 74 aborigines from the

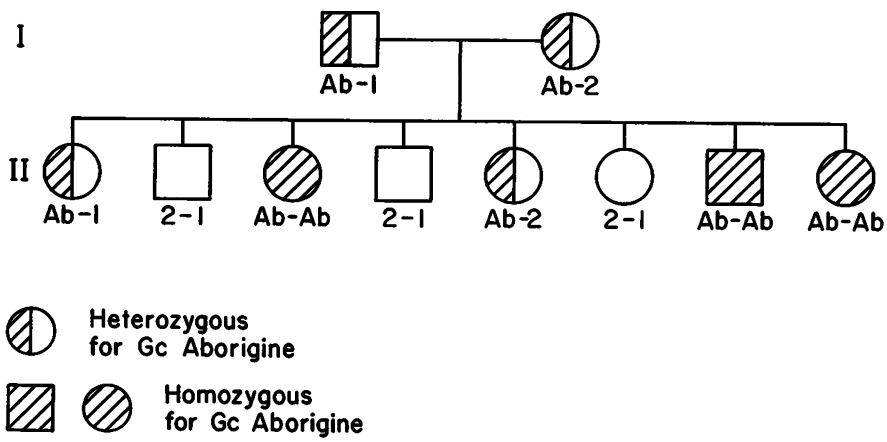

FIG. 5. Inheritance of Gc Aborigine in a kindred from the Cape York area (Aurukun).

TABLE 2. DISTRIBUTION OF GC ALLELES IN A GROUP OF AUSTRALIAN ABORIGINES FROM AURUKUN

	Total	Gc Ab-Ab	Gc Ab-1	Gc Ab-2	Gc 1-1	Gc 2-1	Gc 2-2
Obs.	74*	1	7	1	42	18	5
Exp.	74	0.34	7.41	1.97	40.09	21.35	2.84

Gene Frequencies:
Gc^{Ab} = 0.068
Gc^{1} = 0.736
Gc^{2} = 0.196
χ^2 corrected (Yates) = 1.5888
$P > .20$
*Includes family material.

	Total	Gc Ab-Ab	Gc Ab-1	Gc Ab-2	Gc 1-1	Gc 2-1	Gc 2-2
Obs.	54*	0	4	1	32	13	4
Exp.	53.99	0.11	3.73	1.01	30.37	16.53	2.25

Gene Frequencies:
Gc^{Ab} = 0.046
Gc^{1} = 0.750
Gc^{2} = 0.204
χ^2 corrected (Yates) = 1.6811
$P > .10$
*Corrected for family material.

Aurukun Mission is shown in the upper part of table 2. Since this sample contained family material, a correction was made by excluding all children when a parent was examined, and all sibs except the first sib when more than one was examined. The distribution of Gc types in the remaining sample of 54 individuals is given in the lower part of table 2. The *Gc* frequencies were found to be: $Gc^{Aborigine} = 0.046$; $Gc^1 = 0.750$ and $Gc^2 = 0.204$. The observed and expected distributions, assuming equilibrium, were in close agreement.

DISCUSSION

During the past 3 years information has accumulated rapidly on the distribution of the alleles Gc^1 and Gc^2 in populations from different parts of the world. The observed differences have been reviewed (Cleve and Bearn, 1962). It was found that Gc^1 was more common than Gc^2 in all populations studied. The highest Gc^2 frequencies were 0.34, observed in a sample of 99 Ashkenazic Jews from Israel, and 0.31, found in a sample of 90 Asiatic Indians from Bombay. The lowest frequency of the Gc^2 allele, 0.02, was reported in a Navajo Indian sample of 245 individuals. European populations range from 0.29 in England to 0.23 in Denmark, while the frequency of Gc^2 is significantly lower in African Negroes, where values between 0.10 and 0.05 have been observed. In samples from Chinese and Japanese populations, the frequencies were similar to those in Europeans (0.23 in both populations).

The recognition of variations of the common Gc types in certain Caucasians and African Negroes by Hirschfeld and ourselves suggested the possible existence of additional rare alleles at the *Gc* locus, (Hirschfeld, 1962a and b; Parker, Cleve and Bearn, 1963). The disclosure of two new inherited variations in Chippewa Indians and Australian Aborigines constitutes evidence for a more complex genetic polymorphism of the Gc-system. Both variants are distinguishable from the common group-specific components by their differences in relative electrophoretic mobilities, and both represent variants which are faster migrating than the product of the Gc^1 allele. Results of the family studies discussed above indicate that Gc^{Chip} and Gc^{Ab} behave as alleles of Gc^1 and Gc^2. It seems reasonable to assume that both variants originated by structural mutations of one of the common alleles, more likely by a mutation of Gc^1 than of Gc^2. Although the isolation and partial characterization of the gene products of the alleles Gc^1 and Gc^2 has been achieved recently (Cleve and Bearn, 1962), the methods available at present do not permit their preparation in quantities sufficiently large to determine the precise nature of the structural differences between the group-specific components. It seems probable that the observed variations in electrophoretic mobilities are due to differences in charge rather than to differences in size of the protein molecules.

The characterization of variant types in the Gc-system has been facilitated by application of a modified starch gel electrophoretic technique (Parker, Cleve, and Bearn, 1963). The high resolving power of this procedure permits the recognition of minute differences in relative electrophoretic mobilities which are difficult to disclose by immunoelectrophoresis on agar gel. For example, the starch gel technique has permitted the identification of the variant Gc Chippewa

in the presence of Gc 1 (Gc Chip-1), which was not possible by immunoelectrophoresis. In the screening of large samples for the determination of Gc types in human populations, starch gel electrophoresis appears to be a valuable method. It should be pointed out, however, that Gc Aborigine and other faster migrating variants with a mobility similar to albumin could be overlooked if the investigation is based solely on examination by the present methods of starch gel electrophoresis.

Variations of the Gc-system appear to be characteristic of certain populations or of particular geographic areas. Gc X has been found only in Caucasians and Gc Y only in African and American Negroes. The information on the distribution of the Gc^{Chip} allele is limited. No further populations of the Algonquian linguistic group have been examined and only two other distant Indian populations have been studied. Gc^{Chip} was absent in a sample of 152 sera from Alabama-Coushatta Indians. These two ethnically closely related tribes, both of which belong to the Muskhogean linguistic group, came originally from the Alabama River region and live today as a single unit partly in Texas, where this sample was collected, and partly in Oklahoma. The frequencies found for Gc^1 and Gc^2 were 0.86 and 0.14. Also, in a sample of 245 Navajo Indians no evidence for the presence of Gc^{Chip} was obtained. But in this sample the frequency of Gc^2 was strikingly low (0.02) (Cleve and Bearn, 1961). The sample contained only nine Gc 2-1 phenotypes. Since in our experience with immunoelectrophoresis the occurence of unusual Gc 2-1 phenotypes first draws the attention of the investigator to the possibility of the presence of Gc variants within a population, it is conceivable that faster migrating variants escaped notice in this population.

Studies on the distribution of the allele Gc^{Ab} are discussed in detail elsewhere (Kirk, Cleve and Bearn, 1963). The highest values for Gc^{Ab} were found in the Cape York area, with a gradual decline to the south and west to values of zero or almost zero in the region of the Western Desert. The pattern of distribution of Gc^{Ab} on the Australian continent is in agreement with gene frequency variations observed for other genetic markers and suggests that the mutant has been in existence for a long period of time, before the present distribution of the Aboriginal population on the Australian continent took place. Gc^{Ab} was also found in several sera in a heterogeneous sample from New Guinea, but not in a number of populations from South and South East Asia (Kirk, Cleve and Bearn, 1963). Although more extensive information is needed on the distribution of Gc^{Ab} in Melanesian populations, it may prove to be a particularly useful marker for the study of genetic relationships between Australian Aborigines and populations from New Guinea.

SUMMARY

1. Two genetic variants in the Gc-system have been found. One occurred in a group of Chippewa Indians and has been named Gc Chippewa; the other was first observed in a population of Australian Aborigines and has been termed Gc Aborigine. The variants differ from the usual group-specific components in

their relative electrophoretic mobilities on agar and starch gel. Both variants migrate more rapidly than Gc 1-1.

2. Family studies indicate that the variant phenotypes behave as alleles at the *Gc* locus. They have been designated *Gc*Chippewa (*Gc*Chip) and *Gc*Aborigine (*Gc*Ab).

3. The frequency of *Gc*Chippewa in a sample of 62 unrelated Chippewa Indians was 0.105 and the frequency of *Gc*Aborigine in a population of 54 unrelated Australian Aborigines was 0.046.

Note added in proof. Sept. 7, 1963.

Nerstrøm and Skafte-Jensen (1963) have recently described a gradual transformation of the normal Gc arc in immunoelectrophoresis into a substance with the mobility of an α_1-globulin. The transformation takes place during unfavorable storage conditions of the sample. The patterns obtained by these authors were in certain respects similar to those obtained for the phenotypes Gc Chip-2 and Gc Ab-2. In order to exclude the possibility of such a transformation as the basis of the Chippewa and Aborigine variants, further samples were obtained from 21 selected individuals in the Chippewa population and from five members of the Aborigine pedigree (Fig. 5). These samples were drawn and examined under carefully regulated conditions, and in each case the previous phenotypic classification was verified. These results, together with the large amount of genetic data which has been accumulated, appears sufficient to exclude the possibility that the Chippewa and Aborigine variants can be attributed to storage or other types of non-genetic alterations in the Gc pattern.

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