HETEROGENEITY OF THE INHERITED GROUP-SPECIFIC COMPONENT OF HUMAN SERUM*

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In an earlier communication a method was described by which the three main phenotypes in the group-specific (Gc) system of human serum could be readily identified by starch gel electrophoresis (1). Using this method, differentiation between the Gc types was usually best determined by the appearance of the amido-black stain on the under surface of the gel (the starch-lucite interface). Previous results obtained using a tris-borate buffer were considered insufficiently reliable for routine Gc typing (2, 3). The employment of a buffer system which incorporates lithium (4) has resulted in further improvement in the resolution of the Gc proteins and has disclosed heterogeneity in both Gc 1-1 and Gc 2-2 phenotypes. The similarity between the electrophoretic appearance of the postalbumin bands and the Gc components has been discussed previously (2, 3, 5). Recently, Akfors and Beckman (5, 6) have employed a similar method to determine the Gc phenotypes by starch gel electrophoresis and have reported the existence of heterogeneity of the Gc 1-1 and Gc 2-1 phenotypes. Reinskou (6) using a modified immunoelectrophoretic technique also obtained evidence of heterogeneity in these two phenotypes. Heterogeneity of the Gc 2-2 phenotype was not observed. A preliminary account of some of the results reported in this paper has already been published (7).

Methodology

Vertical starch gel electrophoresis was carried out according to the method described by Smithies (8). Electrophoresis was performed for 5 to 6 hours at 15 v/cm and 4°C in gels of 6 mm thickness. During electrophoresis, two electric fans were placed at the sides of the gel, and the gels were air-cooled throughout the run. A modification of Poulik's buffer incorporating lithium hydroxide was employed (9). Immunoelectrophoresis was carried out in agar gel according to the microtechnique of Scheidegger as modified by Hirschfeld (10). Starch gel immunoelectrophoresis was performed in the manner described by Poulik (11). Purified preparations of the group-specific components were obtained using methods previously reported from this laboratory (12). Anti-Gc serum was prepared in rabbits by immunization with partially purified Gc protein. A contaminating α_1-antitrypsin was identified and then absorbed with purified human α_1-antitrypsin prepared by starch block electrophoresis. The gamma globulin fraction of the resulting specific anti-Gc serum was separated by starch block electrophoresis, concentrated, and titrated before use in the absorption experiments.

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RESULTS

Electrophoretic separation of human serum in the lithium borate buffer system resulted in a greatly improved resolution of proteins migrating between transferrin and the cathodal edge of the albumin. As a consequence the Gc proteins, which in the borate system migrate extremely close to the cathodal edge of the albumin, are more clearly seen. Samples of sera from individuals of type Gc 1-1 show two clear-cut bands of almost equal intensity. The more slowly migrating band which corresponds to the main Gc 1-1 band seen in the borate system is now accompanied by a faster migrating component. In the sera obtained from individuals of type Gc 2-2 two bands are also seen. The main component migrates more slowly than the corresponding component in Gc 1-1 serum and corresponds to the main Gc 2-2 band observed in the borate system. In addition to the main Gc 2-2 component a faster migrating band of less intensity is seen in the position of the main Gc 1-1 band. The electrophoretic difference between the Gc 1-1 and Gc 2-2 phenotypes is illustrated in Fig. 1. It will be noted that the distance between the faster migrating component in the Gc 1-1 and the main Gc 1-1 band is slightly less than the difference between the main Gc 2-2 band and its accompanying faster migrating component. Individuals of phenotypes Gc 2-1 showed three well defined bands in the starch gel system (Fig. 2). Incubation of the sera with neuraminidase prior to electrophoresis did not alter the mobility of the two Gc bands.

The heterogeneity of the Gc components observed in whole serum was observed with purified preparations of Gc 1-1 and Gc 2-2. A sample of purified Gc 1-1 was obtained by methods previously described (12). The penultimate step in the purification procedure consisted of separation of the material on a G-100 sephadex column (Fig. 3). The single symmetrical peak containing Gc specificity was divided into a rapidly and a slowly eluted fraction designated A and B respectively. Each fraction was pooled and concentrated prior to starch gel and starch block electrophoresis. Electrophoretic separation of the concentrated A and B fractions indicated that no separation of the two Gc components had occurred during gel filtration on sephadex. However, the more rapidly eluted fraction (A) contained a small amount of contaminating α_1-antitrypsin. This contaminant was completely absent from the sephadex fraction B.

Comparative starch gel electrophoresis of partially purified Gc 1-1 and Gc 2-2 preparations was performed and indicated a close electrophoretic correspondence between the purified preparations and the appearance of the Gc bands in whole serum (Fig. 4). To exclude the possibility that one of the two bands was unrelated to the Gc system the partially purified preparation was absorbed with purified Gc antiserum with loss of both bands (Fig. 5). In order to obtain additional evidence that both bands observed in the starch gel system possessed Gc

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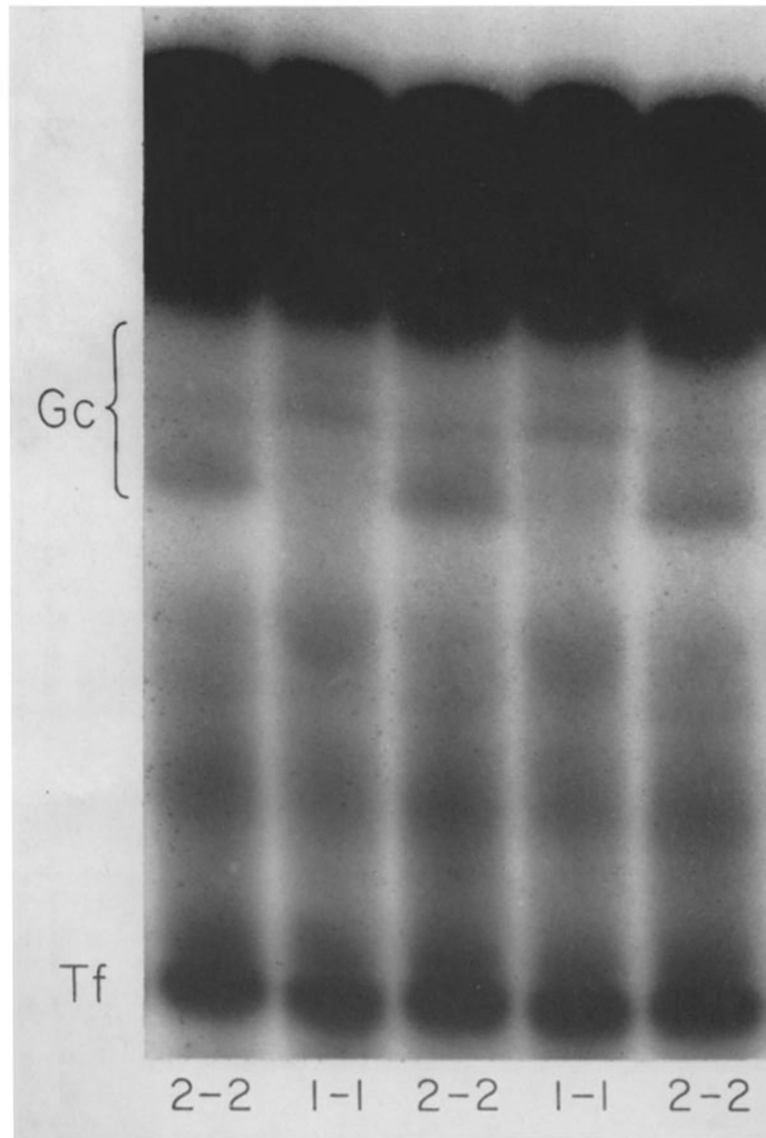

FIG. 1. Vertical starch gel electrophoresis of alternate samples of serum of the two homozygous phenotypes (Gc 1-1 and Gc 2-2) showing two bands in the Gc region. *Tf* indicates the position of the transferrin band.

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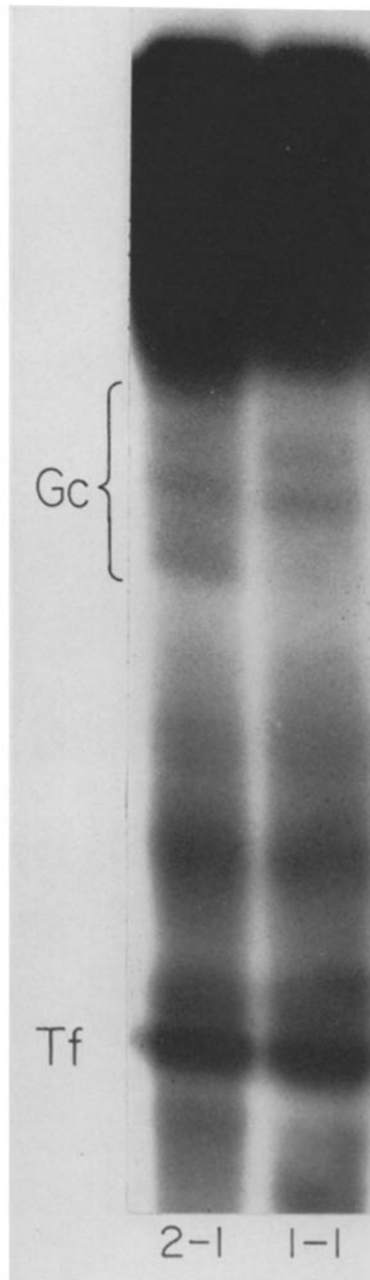

FIG. 2. Comparison of electrophoretic patterns obtained by starch gel electrophoresis of Gc 2-1 and Gc 1-1. Three bands in the Gc region are visible in the heterozygous Gc 2-1 serum whereas only two bands are seen in the Gc 1-1 serum. *Tf* indicates the position of transferrin band.

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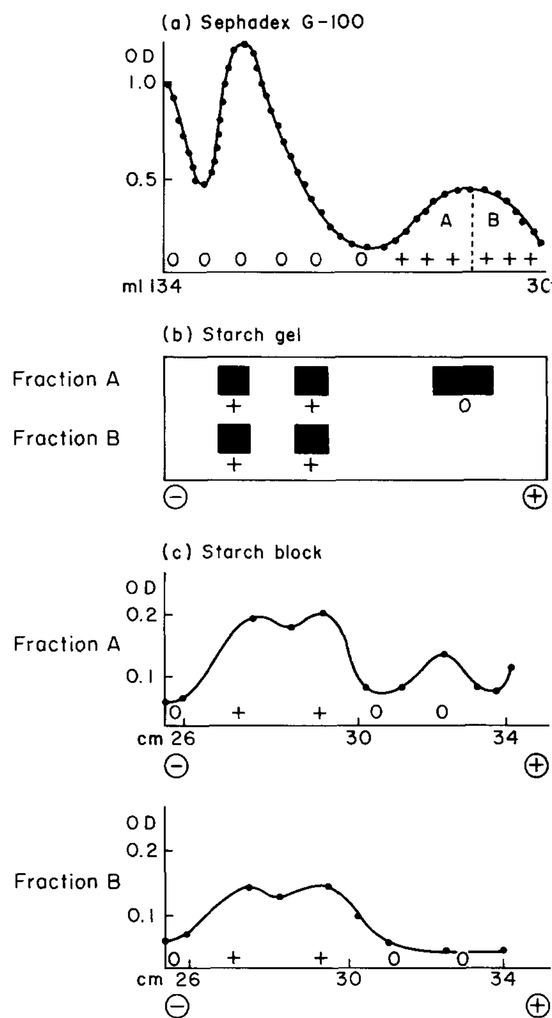

FIG. 3. (*a*) Gel filtration of a Gc 1-1 preparation on sephadex G-100. Flow from left to right.
(*b*) Two fractions A and B from sephadex column, pooled, concentrated, and separated by starch gel electrophoresis.
(*c*) Two fractions A and B from sephadex column separated by starch block electrophoresis. The fast migrating band in Figs. 3 *b* and 3 *c* represents contaminating α_1-antitrypsin. In Figs. 3 *a* to 3 *c* + indicates an immunological reaction with rabbit anti-Gc antiserum.

specificity, a series of punches was made in the starch gel following electrophoresis of serum of types Gc 1-1 and Gc 2-2. The punched out cylindrical starch segments were transferred to immunoelectrophoretic slides and inserted into specially prepared wells in the agar gel, and electrophoresis was performed in the

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conventional manner. Following electrophoresis the antibody trough was filled with specific Gc antiserum (11) and allowed to diffuse toward the separated proteins. A specific precipitation line was observed only in those segments which corresponded to the presumed Gc lines seen in the starch gel system. Using the starch gel immunoelectrophoretic procedure of Poulik (11) employing specific

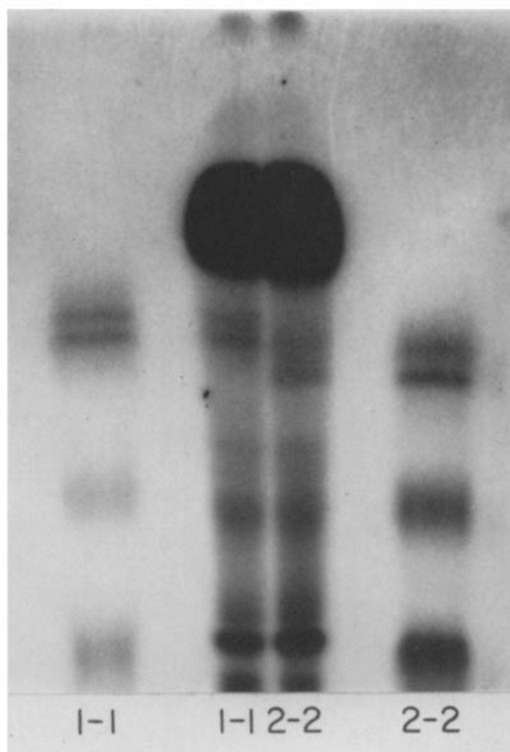

FIG. 4. Comparison of partially purified preparations of Gc 1-1 and Gc 2-2 with their serum counterparts. The mobility of the two bands in the purified preparations corresponds to the two bands visible in whole serum.

Gc antiserum, a prolongation of the precipitin line toward the anode was observed and indicated the presence of Gc specificity in a component migrating ahead of the main band in both Gc 1-1 and Gc 2-2.

In view of the demonstrated heterogeneity of Gc proteins in the starch gel system, it was argued that under conditions of prolonged immunoelectrophoresis an asymmetry of the Gc precipitation arc should be observed and would be characterized by an anodal extension of the precipitation line. Experiments designed to disclose anodal extension of the Gc lines were performed and consisted of prolonged electrophoresis under standard conditions. In several ex-

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periments so conducted a clear-cut prolongation of the Gc precipitation line was observed. The anodal extension of the Gc precipitation line was considerably more marked in sera of Gc 1-1 than in sera of Gc 2-2. In both Gc 1-1 and Gc 2-2 the precipitation line of the faster component was further from the anti-

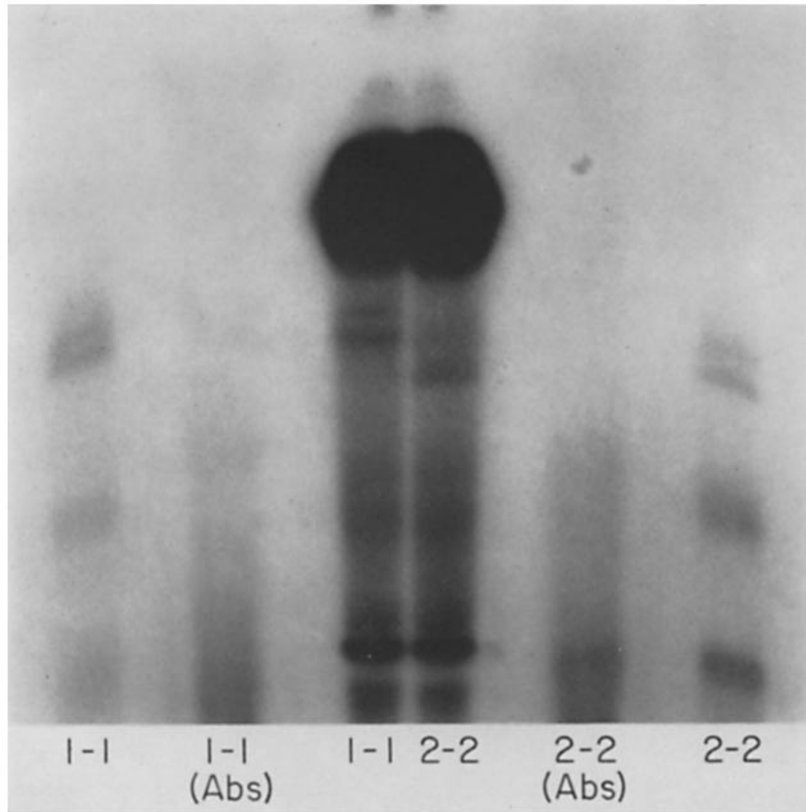

FIG. 5. Electrophoretic comparison of Gc 1-1 and Gc 2-2 with their serum counterparts similar to that illustrated in Fig. 4. Absorption (*Abs*) of the partially purified preparations of Gc 1-1 and Gc 2-2 with specific anti-Gc antiserum removes the two bands seen in the Gc 1-1 and Gc 2-2 positions.

body trough than the slower arc suggesting that the faster component was present in a lower concentration. The extension was quite unlike the denaturation phenomenon described by Nerstrøm in which irregular anodal extension of the Gc precipitin line is also seen (13).

DISCUSSION

The group-specific protein is one of an increasing number of serum proteins that demonstrate structural genetic polymorphism. The variation was first

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recognized by Hirschfeld using an immunoelectrophoretic method (10) but the variation can also be detected by starch gel electrophoresis (1–3). In an earlier communication (1) minor components were visualized which migrated ahead of the principal Gc bands but direct evidence that these components were related to the Gc system was not available. The improved electrophoretic resolution using the lithium buffer system has enabled immunological evidence to be obtained that these components contain the group-specific protein.

The experiments with purified preparations of Gc 1-1 and Gc 2-2 confirm the existence of an additional second component in the Gc system. The possibility that the slower band represented a more slowly migrating polymer was excluded by gel filtration on sephadex G-100. Significant separation of the two components of the Gc 1-1 phenotype was not achieved on this medium, although separation of the two components was easily accomplished by electrophoresis. Successful separation by starch block electrophoresis confirmed the impression gained from the sephadex experiments, that the two components differed in net charge and not in molecular size. Incubation of sera with neuraminidase did not alter the electrophoretic mobilities of the two components and thus provided evidence that the difference in charge between the two Gc components could not be ascribed to a difference in the number of sialic acid residues. It is noteworthy that in some experiments in which whole serum was separated, the distance between the two components of Gc 1-1 was less than the corresponding distance observed with Gc 2-2. When purified preparations of Gc 1-1 and Gc 2-2 were separated the distance between the two components was more closely similar.

At the present time, the molecular structure of the two Gc components in Gc 1-1 and Gc 2-2 is being investigated. It is possible that the main Gc band and the fast band are proteins sharing a common polypeptide chain. The genetic significance of such a shared polypeptide chain is evident from the structural studies on the major and minor components of normal hemoglobin where the existence of a common polypeptide chain is well established. A less likely alternative explanation that cannot yet be excluded is that the fast and slow bands are analogous to the genetically determined variations in cattle transferrin where a single allele appears to control the synthesis of more than one protein (4).

SUMMARY

Heterogeneity of the group-specific (Gc) components in normal human serum has been demonstrated by the use of a lithium borate buffer system in conventional vertical starch gel electrophoresis and by prolonged immunoelectrophoresis in agar gel. In both Gc 1-1 and Gc 2-2 phenotypes a protein component migrates ahead of the main band. Immunological evidence indicates that the faster migrating band contains Gc specificity. The possibility that the two electrophoretically distinct Gc components share a common polypeptide chain is discussed.

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