Determination of Phenotypes in the Human Group-Specific Component (Gc) System by Starch Gel Electrophoresis

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THE TECHNIQUE of starch gel electrophoresis (Smithies, 1955; 1959a) as an analytical method for the resolution of complex mixtures of high molecular weight substances has received extensive application in the study of numerous enzyme and protein systems in human and animal species. By means of specific staining techniques and the use of radioactive markers, it has been possible to visualize individual protein components in the gel. Application of the starch gel technique to the study of human serum proteins enabled Smithies (1955, 1957) to detect hereditary variations in haptoglobin, the α_2-hemoglobin-binding globulin, and in transferrin, the β_1-iron-binding globulin (Giblett, 1962). Resolution of the multiple components in the human transferrin polymorphism was facilitated by electrophoresis under conditions of high voltage and low temperature (Parker and Bearn, 1962), and in the present report a similar technique is described which permits application of starch gel electrophoresis to the determination of phenotypes in another human serum protein polymorphism, the Gc (Group-specific component) system. Inherited variations in Gc were originally detected by the method of immunoelectrophoresis (Hirschfeld, 1959b), and the results of various investigations in this field have been reviewed by Cleve and Bearn (1962). In the present report, conditions of electrophoresis are defined for the resolution of the two common components of the Gc system, and the usefulness of the method is illustrated by the identification of three rare Gc variants. In the adjoining report (Cleve *et al.*, 1963) results are presented of an extensive genetic investigation of two of these variants (*Gc*Chippewa and *Gc*Aborigine). A technique is also described which permits the rapid and routine analysis of 90 samples in a single starch gel, which can be sliced into several layers for the application of appropriate staining techniques.

MATERIALS AND METHODS

Starch: In the course of these investigations, three lots of commercially available starch (Starch Hydrolyzed; Connaught Laboratories, Toronto) were used; with Lot Nos. 165-1 and 176-1, optimum resolution of the Gc proteins was obtained; with Lot 173-1, less satisfactory resolution was observed which appeared to result from the reduced specific advancement of albumin at the

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interface between the gel and the plastic (lucite) supporting mold. This phenomenon is described in detail in the discussion.

The optimum concentration of starch in the gel was 13.0% w/v (Lot 165-1 recommended 12.0% and Lot 176-1, 12.5%). All gels illustrated in the present study were prepared at this concentration. Experiments were carried out to investigate the resolution of the Gc proteins at various starch concentrations from 11 to 15 per cent. At 12.5% starch, the trailing albumin border was slightly more blurred, and the Gc proteins were not as sharply defined as at 13%, although there was a greater separation between Gc 1 and the trailing border of albumin. At 13.5%, conversely, the trailing albumin border was sharper, and Gc 1 migrated closer to this border; similarly, the Gc proteins were not as clear. At 12% starch concentration the effects observed at 12.5% were more accentuated, and at 11% the post-albumins were so blurred that the Gc phenotype could not be distinguished; correspondingly, at 14% starch, the effects observed at 13.5% were accentuated, and at 15% the Gc 1 protein migrated at the trailing albumin border.

Buffer: In all experiments the starch gel was made up in a borate buffer of low ionic strength (.020M H_3BO_3, .0084M NaOH; pH 8.92); a more concentrated buffer (.30M H_3BO_3, .060M NaOH; pH 8.2) was used in the electrode vessels. The more concentrated buffers which were recommended with each lot of Starch Hydrolysed provided less satisfactory resolution of proteins in the post-albumin region as did the tris-borate discontinuous buffer system (Poulik, 1957). By horizontal starch gel electrophoresis in the tris-borate buffer, Schultze *et al.* (1962a; 1962b) have localized a purified Gc 2-1 preparation to the post-albumin region and have shown a clear resolution between Gc 1 and Gc 2 in this sample. These authors also demonstrated the resolution of Gc 1 and Gc 2 in whole serum. However, experiments carried out during the present study have not been able to achieve the necessary reproducibility in the horizontal tris-borate system to permit routine Gc classification of whole serum.

Preparation of Gels: Since the determination of Gc 1 depends upon the relatively enhanced mobility of albumin at the gel-lucite interface, experiments were carried out in an attempt to modify this interface. In routine experiments, a thin layer of mineral oil (Squibb 5592) was spread on the Lucite mold to ease removal of the gel from the mold after electrophoresis. Replacement of the mineral oil with a layer of petroleum jelly (Vaseline), cellophane film (Saran Wrap), or Parafilm caused no change in the observed Gc pattern. Rapid cooling of the hot starch solution in the lucite mold by contact between the bottom of the mold and crushed dry ice did not perceptibly alter the Gc pattern. Slow cooling of the starch in the mold by the circulation of water at 65° C through the bottom of the mold also produced no effect on the Gc separation. No difference in the Gc pattern was observed by normal cooling of the gel at room temperature for periods of 12-24 hours, although after long periods of cooling, the pattern became slightly more blurred.

Conditions of Electrophoresis: Optimum Gc separation was achieved in these experiments by electrophoresis in the vertical starch gel system of Smithies

(1959a) for 3-4 hours at 20 volts/cm and 4° C in gels of 6 mm thickness. During electrophoresis, two electric fans (Hunter-RM model FO12, 1000 CFM) were placed at the sides of each gel, and the gels were air-cooled throughout the run. To facilitate heat exchange, gels were covered with cellophane film instead of petroleum jelly. Water-cooling of the gel molds during electrophoresis was also employed, although it was unnecessary for resolution of the Gc proteins. Additional experiments were carried out with gels of 3 mm thickness at voltage gradients up to 33 volts/cm without improving the resolution of either the Gc pattern or the overall serum protein pattern.

Population Screening: For the survey of large numbers of samples, it has been convenient to use 30-slot inserts (tiers) in conventional (30 x 12 x 0.6 cm) vertical starch gel electrophoresis molds. Each filled slot contains approximately .01 ml sample. By using a double-tiered or triple-tiered gel cover (*i.e.,* a cover designed to hold two or three such inserts), it has been possible to separate 60 or 90 samples on a single gel of normal dimensions. In addition, it has been possible to use 40-slot inserts for the determination of haptoglobin and transferrin phenotypes. After electrophoresis, the gel is routinely sliced into 3 layers of 2 mm thickness each; recent experiments have enabled 4 slices of 1.5 mm thickness to be obtained.

Identification of Gc, Haptoglobin, and Transferrin: Prior to electrophoresis, samples were prepared in the following proportions: 0.1 ml serum: 0.005 ml hemolysate (15% hemoglobin): 0.010 ml Fe^{59} Citrate solution (0.010 mg Fe/ml, 100 μc/ml). After electrophoresis the gel was sliced into three layers The bottom layer was stained for protein with Amido black 10B; Gc phenotypes were determined from the bottom surface of this layer (*i.e.,* from the gel surface adjacent to the lucite mold). Transferrin was determined by exposing the middle layer of the gel against Kodak No-Screen Medical X-ray film for 12 hours; to reduce diffusion of the protein bands, the gel was frozen at −10° C during the exposure. Haptoglobin was determined from the top layer of the gel by the benzidine reagent (0.2 Gm benzidine, 0.5 ml glacial acetic acid, 0.2 ml H_2O_2 (30%) in 100 ml 50% ethanol.

Immunoelectrophoresis was carried out in agar gel according to the microtechnique of Scheidegger as modified by Hirschfeld (Scheidegger, 1955; Hirschfeld, 1959a).

Serum for identification of phenotypes in the Gc system was examined after various periods of storage at −10° C, and no difference was found from the pattern observed in freshly drawn serum. Caucasian and Negro sera were obtained as part of a Gc gene frequency survey. The Chippewa Indian samples are described in the adjoining communication (Cleve *et al.,* 1963).

RESULTS

Identification of Group-specific Components (Gc)

When starch gel electrophoresis was carried out under the described conditions, individual human sera could be classified into three common types according to their protein pattern in the region migrating immediately behind

the broad albumin band. These post-albumin patterns consisted either of a single relatively faster-migrating component, or a single relatively slower-migrating component, or a mixture of the fast and slow components. When these variations were compared with Gc phenotypes established previously by immunoelectrophoresis, it was found that the type with the faster-migrating component in the starch gel corresponded to the phenotype Gc 1-1, the type with the slower-migrating component to Gc 2-2, and the type with both components to Gc 2-1 (Fig. 1). In addition, the identity of these two post-albumin components with Gc 1 and Gc 2 has been demonstrated by comparing the electrophoretic mobilities of purified preparations of Gc 1-1 and Gc 2-2 (Cleve and Bearn, 1962) with standard sera of known Gc phenotype; the purified Gc 1-1 and Gc 2-2 preparations migrated as single components in the starch gel with mobilities corresponding to those of Gc 1 and Gc 2, as indicated in Fig. 1. Over 450 determinations of Gc phenotypes have been carried out on more than 100 different sera representing the three common phenotypes, and in each case there has been complete correspondence between the starch gel and the immunoelectrophoretic phenotypes.

It was found that the serum Gc phenotypes could be determined only from the bottom layer of the gel, and, more particularly, only from the bottom surface of the bottom layer (the starch-lucite interface). The staining pattern with Amido black through the entire thickness of the gel is illustrated diagram-

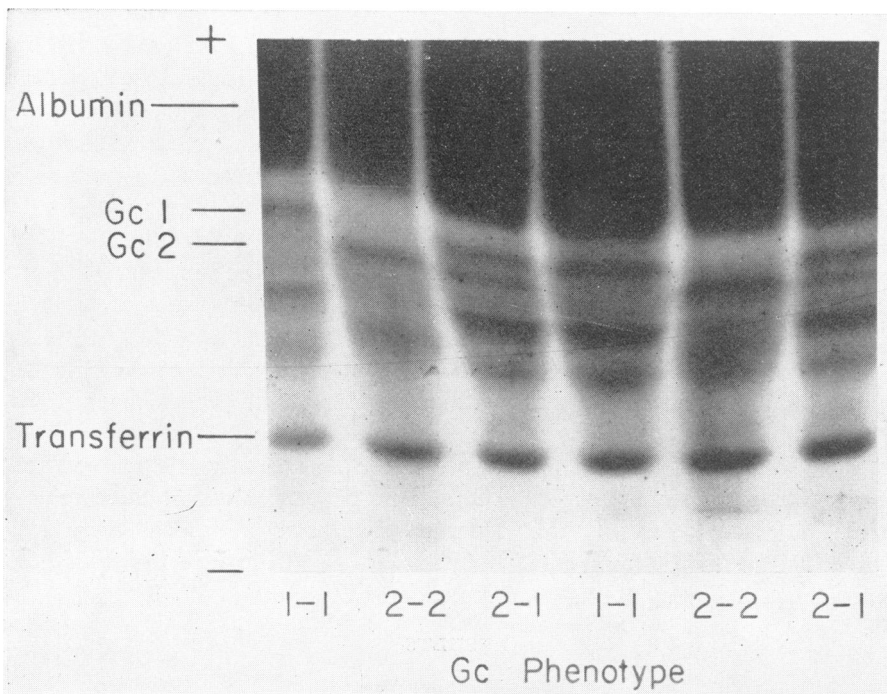

FIG. 1. Common phenotypes of the Gc system by vertical starch gel electrophoresis. Amido-black stain of bottom surface of gel. In this and subsequent photographs of starch gel patterns, only the portion of gel between transferrin and albumin is shown.

matically in Fig. 2. The properties of the starch-lucite interface are such that at this boundary the trailing (cathodal) border of the broad albumin band shows an enhanced mobility relative to the other protein components; the magnitude of this effect is an increase of approximately 20 per cent in the electrophoretic mobility of the cathodal albumin border at the bottom interface. At the top surface of the gel (the starch-cellophane-air interface), there is considerable nonspecific trailing of all protein components. The profile of the

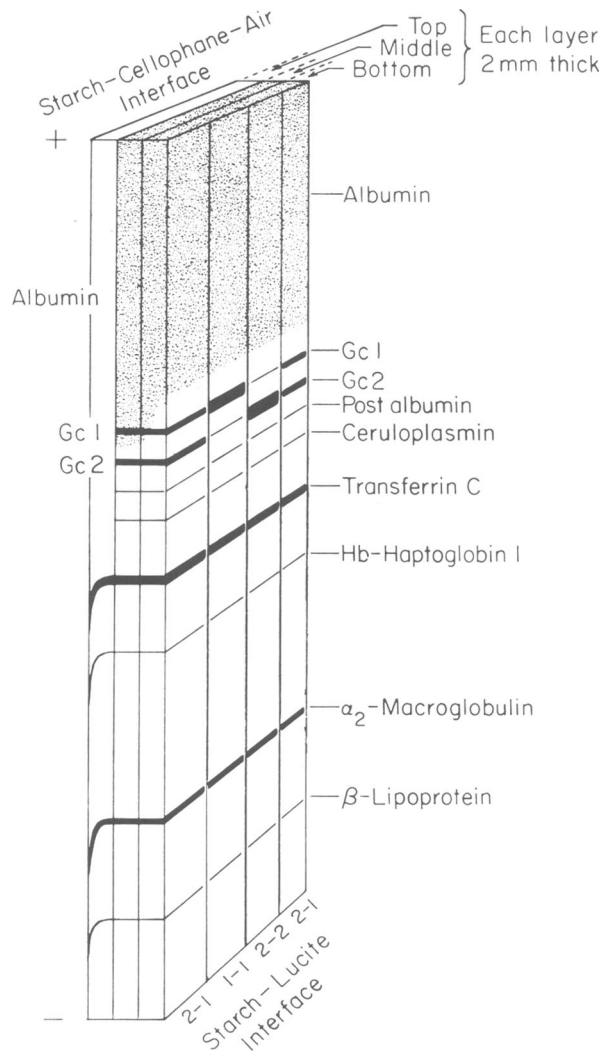

FIG. 2. Determination of phenotypes in the Gc system by vertical starch gel electrophoresis. Diagram of relative mobilities of human serum proteins in cross-section of gel and at bottom surface (starch-lucite interface). All proteins show significant trailing at the top surface (starch-cellophane-air interface), but only albumin shows the enhanced mobility effect at the bottom surface. Other hemoglobin (Hb)-haptoglobin complexes in addition to Hb-haptoglobin 1 are not shown.

trailing edge of the albumin in the cross-section of the gel therefore shows a characteristic "S-like" pattern which is not observed for any other serum protein. The specific increase of the mobility of albumin at the starch-lucite interface is sufficient to pull the trailing border of the albumin region ahead of the Gc components, so that the Gc 1 protein band can be clearly differentiated, whereas in the interior of the gel the Gc 1 band migrates within the albumin region and cannot be distinguished.

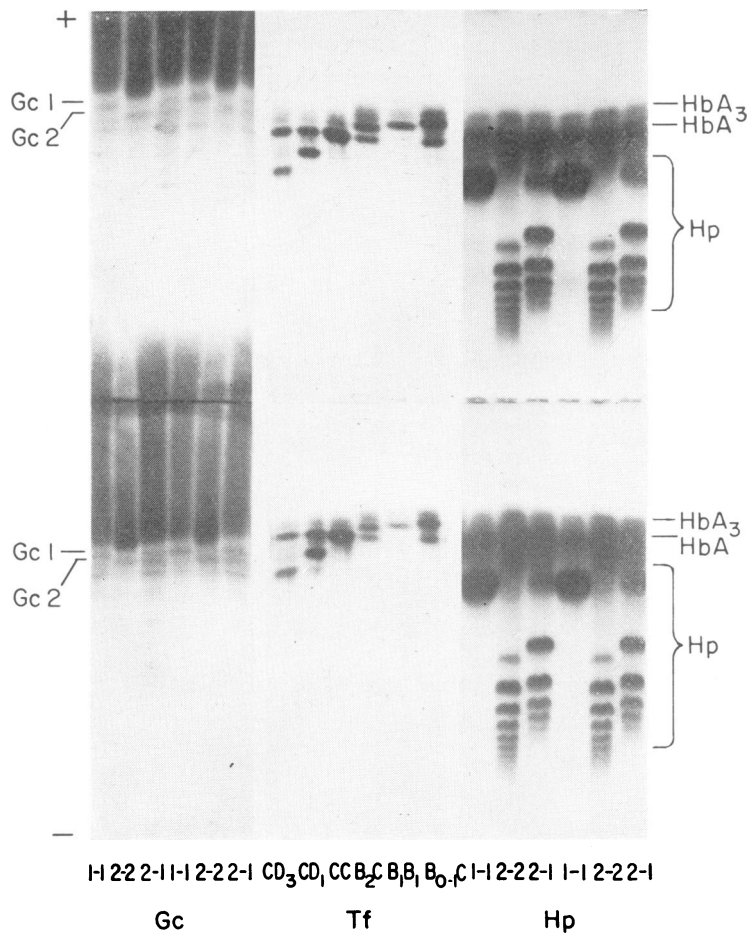

FIG. 3. Population screening of serum proteins by starch gel electrophoresis. Gels prepared in conventional vertical electrophoresis mold with cover containing two sample-insert tiers of 16 slots each. Thus, 32 samples were analyzed on these gels. After electrophoresis gels were sliced into three layers:

(left) Amido-black stain of bottom surface of gel, indicating Gc phenotypes.

(center) Autoradiograph from middle layer of gel, indicating transferrin (Tf) phenotypes. Fe^{59} added to samples prior to electrophoresis.

(right) Benzidine stain of top layer of gel, indicating haptoglobin (Hp) phenotypes. Hemoglobin added to samples prior to electrophoresis. In addition to the hemoglobin-haptoglobin complexes, excess normal adult hemoglobin (HbA_1) and a hemoglobin decomposition product (HbA_3) are also observed.

Measurement of the potential gradient along the gel at one cm intervals during electrophoresis revealed a relatively sharp peak of potential (25 volts/cm) which migrated at the trailing border of the albumin; all other portions of the gel showed approximately the same potential (20 volts/cm) throughout the run. Smithies (1955) has described similar variations in potential at lower voltage gradients (5-6 volts/cm).

Simultaneous Large-Scale Determination of Gc, Transferrin and Haptoglobin Phenotypes

Application of the high voltage technique to the screening of large numbers of sera is indicated in Fig. 3. In this experiment a double-tiered gel cover, with 16 sample slots in each tier was used, which permitted 32 sera to be analyzed in the gel. The bottom layer of the gel was stained with Amido black for the determination of Gc phenotype, and the other two slices were used for the detection of transferrin and haptoglobin. To illustrate the resolution of a variety of Gc, Tf, and Hp phenotypes, the experiment in Fig. 3 represents a composite illustration from three different starch gel experiments in which the appropriate layer was taken for each protein component. Inserts of 30 slots have now been employed in the system, with the result that 90 samples can be analyzed in a single gel. With multiple-tier gels, the albumin region from one insert series migrates into the haptoglobin region of the adjacent anodal insert series, but this effect has not interfered with the determination of haptoglobin phenotypes.

Identification of Chippewa, Caucasian, and Negro Variants in the Gc System

By the use of the high voltage system, it has been possible to resolve three Gc variants in addition to Gc 1 and Gc 2, which are the components common to all populations (Cleve and Bearn, 1961). The existence of these variants was originally suggested by slight alterations from the normal immunoelectrophoretic patterns observed during a survey of Chippewa Indian, Caucasian, and American Negro populations. The immunoelectrophoretic patterns of these variants are illustrated in Fig. 4. In the case of the Chippewa variant, by immunoelectrophoresis the double arc of the Chippewa sample was more pronounced than in the common Gc 2-1 phenotype. Examination of the serum by starch gel electrophoresis revealed the pattern shown in Fig. 5. The Chippewa phenotype contained two prominent components in the Gc region; the electrophoretic mobility of the slower-moving component coincided with that of Gc 2, whereas the mobility of the faster-migrating component was approximately intermediate between Gc 1 and the trailing border of the albumin region. An investigation of the inheritance and gene frequency of this variant is presented in the adjoining report (Cleve, *et al,* 1963); as described there, it has not yet been possible to distinguish the Chippewa variant from Gc 1 by immunoelectrophoresis.

The immunoelectrophoretic pattern of the Caucasian variant (Fig. 4) revealed a double arc which was slightly less extended toward the Gc 2 region than in the normal Gc 2-1 phenotype; a similar variant phenotype (Gc 1-X) has been

described by Hirschfeld (1962) in a Swedish and Norwegian individual. By starch gel electrophoresis (Fig. 6) it was observed that the Caucasian phenotype consisted of a faster-moving component with the mobility of Gc 1 and a slower-migrating component with a mobility slightly greater than that of Gc 2. The Caucasian phenotype has been found in only one individual.

Finally, in the Negro phenotype, which has been observed in three unrelated individuals, the immunoelectrophoretic pattern (Fig. 4) revealed a more pronounced double arc than is present in the normal Gc 2-1 phenotype, although not as prominent as in the Chippewa phenotype; Hirschfeld (1962) has observed a similar phenotype (Gc 1-Y) in an African Negro. By starch gel electrophoresis (Fig. 7) the Negro sera revealed a slower-moving component with the mobility of Gc 2 and a faster-migrating component with a mobility slightly slower than Gc 1. The observed patterns in the Negro phenotype were found to be reproducible by both immunoelectrophoresis and starch gel electro-

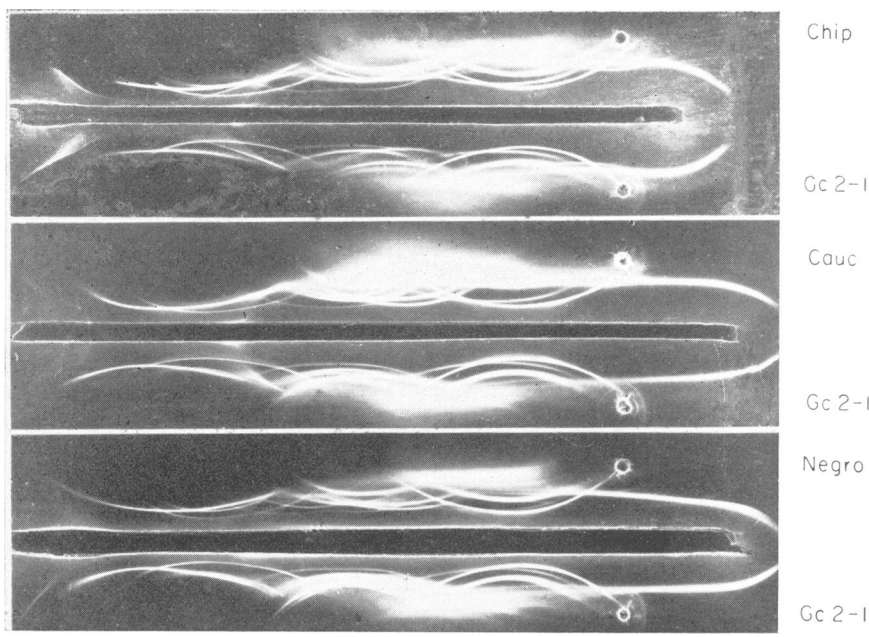

FIG. 4. Identification of variant phenotypes in the Gc system by immunoelectrophoresis of human serum. The Gc precipitin lines are the faint arcs lying closest to the antibody troughs in the middle portion of each pattern.

(top) Comparison of standard Gc 2-1 serum with a variant serum from a Chippewa Indian (Chip.). The more pronounced double arc in the variant pattern indicates the presence of a Gc protein migrating more rapidly than Gc 1 (see Fig. 5).

(center) Comparison of standard Gc 2-1 serum with a variant serum from a Caucasian individual (Cauc.). The less cathodally-extended double arc in the variant pattern indicates the presence of a Gc protein migrating more rapidly than Gc 2 (see Fig. 6).

(bottom) Comparison of standard Gc 2-1 serum with a variant serum from a Negro individual. The more pronounced double arc in the variant pattern indicates the presence of a Gc protein migrating more rapidly than Gc 1 (see Fig. 7, where, however, the Negro variant migrates more slowly than Gc 1.)

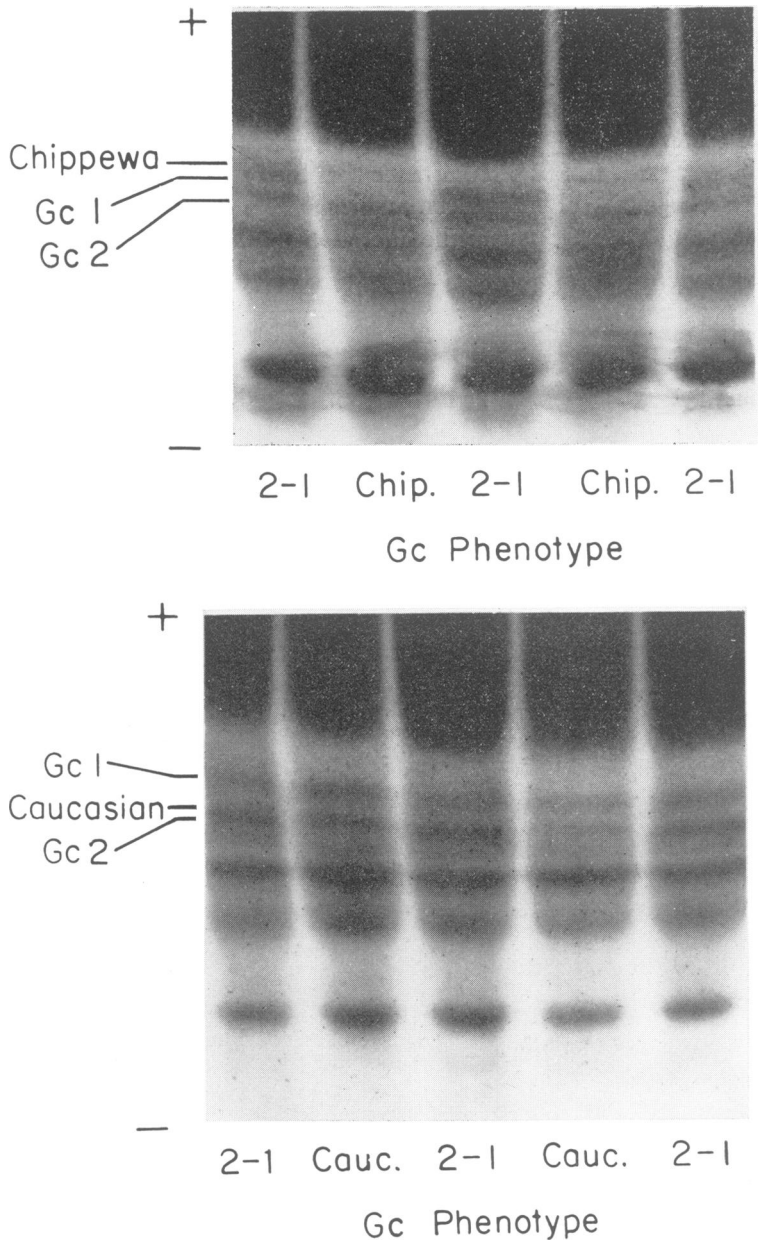

FIG. 5. Identification of Chippewa variant by starch gel electrophoresis. Comparison of standard Gc 2-1 serum with serum from a Chippewa Indian (Chip.). The Chippewa Gc band, which has a mobility intermediate between Gc 1 and albumin, appears to correspond to the more rapidly migrating component observed by immunoelectrophoresis (Fig. 4, top).

FIG. 6. Identification of Caucasian variant by starch gel electrophoresis. Comparison of standard Gc 2-1 serum with serum from a Caucasian individual (Cauc). The Caucasian Gc band, which has a mobility slightly greater than Gc 2, appears to correspond to the more rapidly migrating component observed by immunoelectrophoresis. (Fig. 4, center).

phoresis for repeat samples obtained from each of the three individuals after periods of two to four months. A similar variant phenotype was observed in serum from a brother of one of the propositi. If, as appears likely, the faster migrating Negro component represents an inherited variation in the Gc system, then the mutation can be localized to an alteration in the Gc^2 rather than the Gc^1 gene, since it is in this circumstance that the relative electrophoretic mobilities by starch gel and immunoelectrophoresis are most easily reconciled. If the mutation were in the Gc^1 gene, the alteration would require the variant to migrate more rapidly than Gc 1 by immunoelectrophoresis and more slowly than Gc 1 by starch gel electrophoresis; if the mutation is in the Gc^2 gene, the alteration in mobility is in the same direction by both analytical techniques, although for reasons that are not clear the alteration is relatively greater by immunoelectrophoresis.

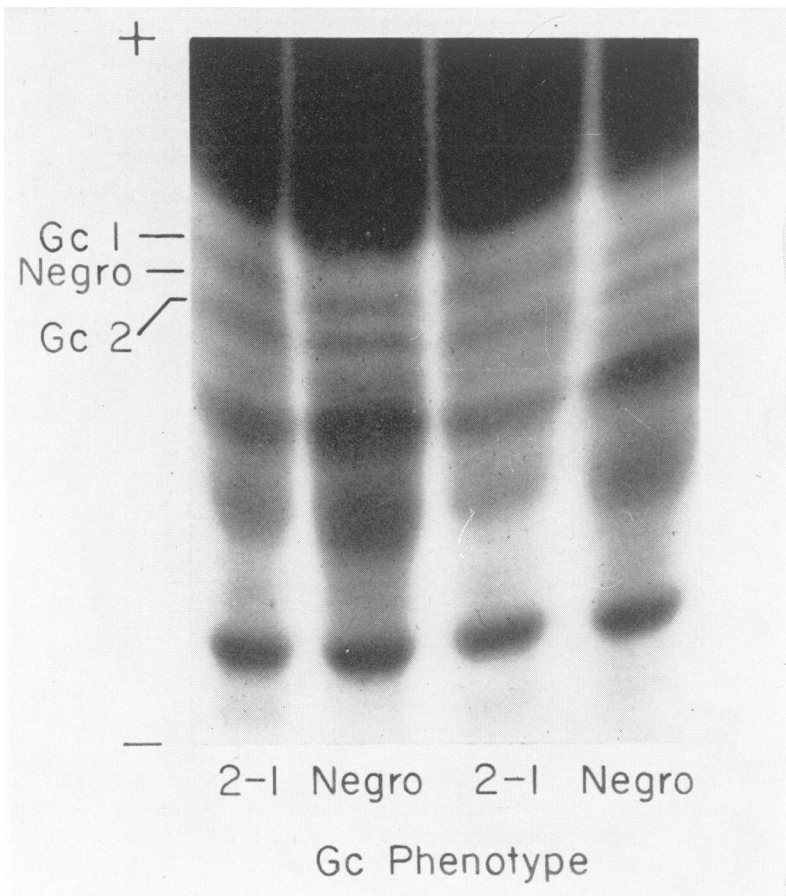

FIG. 7. Identification of Negro variant by starch gel electrophoresis. Comparison of standard Gc 2-1 serum with serum from a Negro individual. The Negro Gc band migrates slightly more slowly than Gc 1 in the starch gel pattern, although by immunoelectrophoresis it migrates more rapidly than Gc 1 (Fig. 4, bottom).

DISCUSSION

The Selective Enhancement of the Mobility of Albumin

An important aspect of the application of starch gel electrophoresis to the determination of Gc phenotypes is the unique property of the starch-lucite interface which makes this identification possible. Resolution of the Gc 1 protein band and of such faster-moving Gc proteins as the Chippewa variant is achieved only at the bottom surface of the starch gel adjacent to the lucite mold, and depends upon the selective enhancement of the mobility of albumin relative to the post-albumin components, among which are the Gc proteins. Under ordinary conditions of electrophoresis (5 volts/cm, 18 hours, 20° C), Gc 1 migrates within the albumin region. Under the high voltage conditions, (20 volts/cm, 3-4 hours, 4° C), the cathodal border of the albumin band is sufficiently increased in mobility at the lucite interface to achieve the differentiation not only of Gc 1 but also of other Gc variants, such as the Negro variant, which migrates in the Gc 1 region, and even of the Chippewa variant, which has a mobility approximately intermediate between Gc 1 and albumin. The selective advancement effect is absent in interior planes of the gel; within less than 1 mm from the bottom surface of the gel, the Gc 1 component and all faster-migrating post-albumins migrate within the albumin region. Thus, on interior planes of the gel, which are the commonly visualized surfaces after staining, Gc 2 is the component migrating closest to the albumin.

The selective advancement of albumin at the starch-lucite interface is difficult to interpret. The failure of various alternative mold surfaces to modify the observed pattern indicates that the effect is not specific for the lucite surface. It is unlikely that phenomena such as temperature dependence of mobility are responsible for this pattern. A temperature gradient exists across the thickness of the gel, since almost all of the heat transfer occurs through the air-cooled surface of the gel and not through the lucite surface; however, the temperature-dependence of albumin mobility would necessarily be remarkably different from that of other serum proteins in order to produce the observed mobility increment at the bottom surface, since no other serum protein shows a tendency for such an increased mobility. It is also unlikely that the albumin effect is caused by concentration dependence of mobility. In an experiment on the serial dilution of a serum sample the selective advancement of albumin was observed at dilutions as high as 1:64. Two tentative explanations may be offered for the albumin effect:

1. It is possible that the porous structure of the gel at the bottom surface is sufficiently increased that the molecular-sieving action of the gel is modified. A small increment in porosity could permit albumin, with a sedimentation coefficient of 4.6 S, to migrate more easily through the gel, whereas the effect may be negligible for smaller molecules such as Gc (4.1 S), which may already be moving relatively freely through the gel. The observed alterations in the relative mobilities of albumin and Gc 1 at various starch concentrations from 11 to 15 per cent may be attributed to such changes in gel porosity. However, it is unlikely that the enhanced mobility of albumin at the lucite interface results

from a change in porosity at the interface. The observation that no serum protein except albumin shows such a mobility increment requires that the effect be highly specific and operate over a narrow molecular weight range, since transferrin (MW = 83,000), haptoglobin-1 (MW = 80-100,000) and hemoglobin (MW = 68,000) fail to exhibit even a slight increase of mobility at the bottom surface. Considerable variation in the preparation of the gel failed to alter the observed pattern and suggests that the effect cannot easily be attributed to a modification of the gel structure.

2. Alternatively, the selective mobility increment of albumin may indicate that chromatography is taking place in the starch gel in addition to separation based on molecular charge and size. Since the charge structure at the lucite interface is undoubtedly different from that within the gel, it is likely that chromatographic separation would also be affected at the interface. The altered chromatographic relationship would then be specific for albumin, since the interface mobility of other serum proteins is not affected. It is possible that chromatographic retardation of albumin within the starch gel may be significantly decreased at the lucite interface, so that the mobility of the protein at the interface is increased. It is clear, however, that the precise explanation for the selective advancement of albumin at the starch-lucite interface must await further experimentation.

Relationship of the Gc Proteins to Other Post-Albumin Components in the Starch Gel Pattern

From the starch gel patterns obtained in the present study (Figs. 1, 5, 6 and 7) it is seen that between transferrin and the trailing border of the albumin there are four prominent components which can be resolved in all sera. In order of increasing mobility, these components are: ceruloplasmin, an unidentified post-albumin band, Gc 2, and Gc 1. Smithies (1959a) has demonstrated individual variations in the post-albumin region of whole serum after electrophoresis in borate buffer; comparison of these serum patterns with those of the present study indicates that the variations of Smithies are probably not related to the Gc system. The possibility that such variations may represent an additional heterogeneity in the post-albumin region is complicated by the likelihood that the faster-moving component in the variations observed by Smithies is the Gc 2 band, whereas the slower-moving component is probably unrelated to Gc.

In addition to the four principal components resolved between transferrin and albumin, a post-albumin band which migrates immediately behind Gc 2 has been observed in some sera; this component is clearly seen in the serum of the Chippewa individual (Fig. 5). A splitting of the protein band in the ceruloplasmin position into two components has also been observed, and further studies are in progress to clarify this heterogeneity.

Determination of Gc Phenotypes by the Starch Gel Technique

The use of starch gel electrophoresis for the determination of Gc phenotypes presents certain advantages over the immunoelectrophoretic method, such as the

simultaneous analysis of numerous samples and the reduction in time required for the determination. In addition, the starch gel method should facilitate the identification of Gc variants, since relative electrophoretic mobilities are more easily compared in the starch gel system (*e.g.*, the elaborate polymorphism of human transferrin: Parker and Bearn, 1962) than by immunoelectrophoresis. The similarity between the immunoelectrophoretic patterns obtained for the variant Caucasian and Negro phenotypes in the present study to those observed by Hirschfeld (1962) makes it likely that the characteristic protein components in the starch gel patterns (Figs. 6 and 7) correspond, respectively, to the X and Y components in the Hirschfeld classification of Gc variants. It is also possible that the combination of immunoelectrophoretic and starch gel techniques will increase the scope of experiments directed at the elucidation of the presently unknown biological role of the Gc system.

The identification of the Gc proteins depends partly upon the intensity of the Gc bands relative to the intensities of the other post-albumin components. In sera of phenotype Gc 2-2, a faint component is present which migrates in the Gc 1 position; correspondingly, in sera of Gc 1-1 phenotype, a faint component is present which migrates in the Gc 2 position. In the following communication (Cleve, *et al.*, 1963) a weak post-albumin component, apparently unrelated to the Gc system, is described which migrates in the position of the Chippewa variant. These minor components have not been identified, although certain α_1- and α_2-globulins are known to migrate in the post-albumin region of the starch gel (Cleve and Bearn, 1962). Possible confusion in Gc typing of serum may occur if one of the faint components is selectively increased; for example, a Gc 2-2 serum could be interpreted as Gc 2-1. However, the large number of sera which have been compared by immunoelectrophoresis and starch gel electrophoresis suggests that this possibility is unlikely. A similar difficulty could arise in the identification of a hypothetical Gc variant migrating more slowly than Gc 2, since in such a case, the Gc band could be obscured by the slower-moving post-albumin component which is not part of the Gc system, but which has an intensity approximately equal to that of the Gc proteins. However, in the large number of sera which have now been examined by immunoelectrophoresis in many laboratories, no example of a variant migrating more slowly than Gc 2 has been found. A further potential difficulty in identification of Gc proteins by starch gel electrophoresis is the possibility that certain variants may migrate within the albumin region. In the adjoining report (Cleve *et al.*, 1963) a variant is described in an Aborigine population which by immunoelectrophoresis appears to migrate slightly more rapidly than the Chippewa variant. The starch gel patterns of individuals homozygous by immunoelectrophoresis for the variant reveal no definite Gc band. Punch experiments described in that report indicate the presence of a Gc component within the trailing portion of the albumin region. It therefore appears that the most reliable method for determination of Gc phenotypes is by a conjunction of the starch gel and immunoelectrophoretic techniques. Under present conditions, neither method alone is capable of detecting all of the known phenotypes.

The increased capacity of the gel for rapid determination of multiple serum

phenotypes is of interest in human genetics when relatively large numbers of individuals are available for population studies at the biochemical level. Results of the present study indicate that it is feasible for one person using three gels to screen 270 samples of human serum per day by starch gel electrophoresis. The practicability of slicing each gel into four layers permits simultaneous analysis of the samples by diverse enzyme and protein stains.

SUMMARY

A method is described for the determination of Gc (group-specific component) phenotypes by starch gel electrophoresis of human serum. By electrophoresis under conditions of high voltage and low temperature, the Gc 1-1, Gc 2-1, and Gc 2-2 phenotypes are readily identified. The method has also been applied to the identification of variants in the Gc system in addition to Gc 1 and Gc 2; thus, characteristic post-albumin components which correspond to variant Gc phenotypes observed by immunoelectrophoresis have been found in Chippewa Indians and in Caucasian and Negro populations.

Detection of Gc bands with a mobility equal to or greater than that of Gc 1 is dependent upon a specific enhancement in the mobility of albumin at the interface between the gel and the plastic supporting mold; the effect is consistently observed under high voltage conditions in borate buffer, and enables rapid and reproducible identification of Gc types to be made.

A method is also described which permits the convenient analysis of large numbers of samples in a single gel, which can be sliced into multiple layers for utilization of specific staining techniques.

Note added in proof. Sept. 7, 1963.

Since this paper was completed Nerstrøm and Skafte-Jensen (1963) have reported that under certain unfavorable storage conditions, the normal Gc pattern may be modified to produce a more rapidly migrating arc in immunoelectrophoresis. As discussed in the adjoining report (Cleve *et al.*, 1963), it is extremely unlikely that such a transformation is responsible for the Chippewa variant. It is also unlikely that such an effect could account for the Negro variant, since the Negro phenotype could be clearly identified by starch gel electrophoresis. Those sera in the present experiments in which immunoelectrophoretic alterations similar to those of Nerstrøm and Skafte-Jensen could be induced by unfavorable storage conditions revealed no Gc component corresponding to the Negro variant in the starch gel system.

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