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Comparison and Combination of the Starch-Gel and Filter-Paper Electrophoretic Methods Applied to Human Sera: Two-Dimensional Electrophoresis

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Recently a method of zone electrophoresis using starch gel as the supporting medium has been described (Smithies, 1955*a*, *b*), which enables a number of serum-protein components to be demonstrated which are not seen with the classical electrophoretic methods. A series of experiments has therefore been carried out in order to correlate the results obtained with starch gels with those obtained with filter-paper electrophoresis. The results of these experiments suggested that a suitable combination of the two methods in a single method would lead to a more complete resolution of the serum-protein components than is possible by any other single method. A system involving electrophoresis in two dimensions, first on filter paper and secondly at right angles in starch gel, has been developed. This two-dimensional system has been briefly described in a preliminary report (Smithies & Poulik, 1956). A full account of the method is given below, and the results obtained with it are discussed.

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A small number of abnormal sera have been studied by the starch-gel and filter-paper electrophoretic methods in order to confirm the findings with normal sera, and to illustrate the potentialities of starch-gel electrophoresis in the study of abnormal sera.

EXPERIMENTAL

Starch-gel electrophoresis

The method of starch-gel electrophoresis described by Smithies (1955*b*) was used. No important changes in the procedure have been made, but the method of preparing a starch suitable for serum electrophoresis has been studied in more detail. The source of unhydrolysed potato starch appears to be of considerable importance. Of the many varieties of potato starch tested, starch obtained from Denmark gives a product closest to that originally used. Starch from Idaho was also found to be suitable. In testing a given starch, hydrolysis is carried out with acetone–hydrochloric acid (1 vol. of conc. hydrochloric acid to 100 vol. of reagent-grade acetone). Several small samples of the starch and the acetone–hydrochloric acid are equilibrated overnight in a thermostatically controlled room at 37°. The hydrolysis is started by adding the acetone–hydrochloric acid to the starch samples (approx.

60 ml./30 g. of starch). The reaction is stopped by adding 15 ml. of aq. M-sodium acetate. Thorough washing with water and drying with acetone follow. A range of hydrolysis times is tried (usually ½, 1, 1½, 2 and 2½ hr.) and the hydrolysis time giving the most suitable product is used for preparing larger quantities. If necessary the hydrolysis time is more closely defined by repeating the test with narrower time intervals. It is redetermined whenever a new batch of starting material is used. The selection of the most suitable hydrolysis time is made by considering the mechanical properties of the gel obtained from the hydrolysed starch at a concentration of 14 g./100 ml. of buffer solution. Considerable under-hydrolysis gives a product from which a solution cannot be made even after prolonged heating of the starch suspension. A moderately under-hydrolysed starch will go into solution only with difficulty and sets to a sticky gel which will stretch considerably under tension. A correctly hydrolysed starch will set to a firm gel which has good mechanical strength yet under tension breaks cleanly. Over-hydrolysis leads to progressively weaker gels. When a suspension of correctly hydrolysed starch is heated during the preparation of the gel a temperature is reached at which the suspension becomes semi-solid. However, further heating will cause the semi-solid mass to liquefy. This temporary semi-solid consistency should not be confused with the final consistency of an under-hydrolysed starch, which will persist even after prolonged heating.

When a starch with satisfactory mechanical properties at a concentration of 14 g./100 ml. of buffer solution has been obtained the conditions for electrophoresis are adjusted to the optimum by varying the concentration of the borate buffer solution used in making the gels. A stock solution of buffer is made containing 0·5 mole of boric acid and 0·2 mole of sodium hydroxide/l. Dilute solutions are prepared from this stock solution ranging from 0·020 to 0·035 M in total borate. The final concentration selected is that which gives a serum-protein pattern in which the front and back of the albumin zone are of approximately equal sharpness. Increasing the concentration of borate leads to a sharpening of the front of the albumin zone.

Filter-paper electrophoresis

A conventional system which has been used for several years by one of us (M. D. P.) was employed. Whatman no. 1 filter-paper strips, 4 cm. × 35 cm., are used with a buffer solution (pH 8·55) of the following composition: sodium acetate, 0·048 M; sodium barbital, 0·048 M; hydrochloric acid, 0·0073 M. Four strips are set up simultaneously. The strips are freely suspended in a horizontal plane, supported at the ends by a removable plastic framework. The whole system of electrode vessels and strips is placed in a closed chamber (30 cm. × 25 cm. × 10 cm.) so that excessive drying does not occur during electrophoresis. Serum (0·01 ml.) is applied to each strip as a narrow zone. Electrophoresis is carried out for 16 hr. with a potential across the electrodes of 115–120 v. After the electrophoresis the strips are dried at room temperature and the proteins are stained with Amido-black 10 B (Bayer Leverkusen, Germany).

The distributions of the stained proteins on the filter-paper strips shown in Plates 5 and 6 were obtained by photographing the tracings on the cathode-ray tube of an electronic computer previously described (Pinteric & Poulik, 1954; Poulik & Pinteric, 1955).

Combination of the starch-gel and filter-paper electrophoretic methods

The following combination of the two electrophoretic techniques has been successfully employed.

Serum (0·1 ml.) is subjected to electrophoresis on a Whatman no. 3 filter-paper strip (8 cm. × 35 cm.) for 16 hr. with the buffer solution, voltage gradient and general experimental arrangement described above. After the electrophoresis the paper is removed from the moist chamber and narrow strips are cut from the two edges. These strips are stained in a rapid manner with bromophenol blue while the unstained portion of the filter paper is stored in the cold room. The stained strips are then used as guides to that part of the unstained filter paper over which the serum proteins are distributed. Serial sections (5 mm. × 14 mm.) are cut from this part of the filter paper, as illustrated in Fig. 2. Each section is then introduced into a slit in a starch gel alongside a small piece of filter paper (5 mm. × 5 mm.) soaked in the original serum. With care, intermixing of the two samples can be avoided. The remaining part of the original filter-paper strip is later stained with Amido-black 10 B to provide an accurate index of the location of the protein fractions in the serial sections. After the second electrophoresis in the starch gels, slicing and staining are carried out in the usual way. Protein components common to both the whole serum and the serum fraction obtained from the filter-paper electrophoresis can then be identified as uninterrupted bands across the whole width of the gels. If the filter-paper section removed from the gel is also stained at the end of the experiment any protein failing to enter the gel can be detected.

Two-dimensional electrophoresis

Serum (0·01–0·015 ml.) is first subjected to electrophoresis at room temperature (25°) on 5 mm. × 350 mm. strips of Schleicher and Schüll 598 YD filter paper (Carl Schleicher and Schüll, Dassel, Germany) for 16–18 hr. with a potential of 115–120 v between the electrodes and the buffer solution described above. Several of these strips are suspended in the closed chamber, the humidity of which is controlled by placing alongside the narrow filter-paper strips wider strips soaked in the same buffer solution but with no serum applied to them. One of the narrow strips is stained to check the separations obtained at the end of the filter-paper electrophoresis before the second starch-gel electrophoresis.

The technique used for the starch-gel electrophoresis, which is carried out at right angles to the filter-paper electrophoresis, is an extension of that previously described for small gels, the only change being in size; suitable dimensions for the gel are 16 cm. × 12 cm. × 0·6 cm. A cut is made across the 12 cm. dimension of the gel about 5 mm. from one end with suitably mounted razor blades so that a small strip can be removed from the end of the gel. A second parallel cut is made 2 or 3 cm. from the first and the gel is displaced into the space left by the removed portion in order to open up a slot. Into this slot is inserted the protein-bearing portion (less than 12 cm. in length) of the narrow filter-paper strip. The previously stained strip enables this portion to be selected, and the manipulations can be facilitated by picking up the protein-bearing filter paper with plastic spring clips mounted parallel to each other and spaced about 11·5 cm. The slot in the gel is then

closed up around the inserted strip by returning the displaced gel to its original position. The removed portion of gel is replaced and a coating of melted petroleum jelly is applied to the exposed gel surface to prevent evaporation during the electrophoresis. Electrical contact with the ends of the gel is made with filter-paper wads soaked in bridge solution, and a voltage gradient of 6 v/cm. is applied for 6 hr. The gel is then removed from the tray and placed on a cutting block ready for slicing in a horizontal plane. The filter-paper strip is removed before the slicing, which is done with a dermatome knife blade supported on either side of the gel by plastic guide strips (3 mm. thick) attached to the cutting block. The knife must be free from any irregularities in its cutting edge, and slicing is done most easily if a piece of thick plastic sheet of the same dimensions as the gel is allowed to rest on top of it during the slicing. The knife should be carried through the gel in a single uninterrupted movement. The proteins are stained with Amido-black 10B in the usual way. If the filter-paper strip removed from the gel is also stained any protein failing to enter the gel can be detected and its position relative to the other components can be determined.

Gel photographs

The photographs shown in the plates were taken on Adox KB17 35 mm. film (Dr C. Schleussner Fotowerke G.m.b.H., Frankfurt-on-Main, Germany) with Tungsten illumination and a Wratten A25 red filter. Development was for 12 min. at 20° with Adox E10 developer (resulting contrast equivalent to a photographic gamma of approximately 0·65). This type of film and development give a close representation in the negatives and final enlargements of the wide range of intensities of dye seen in the starch gels.

Figs. 2–4 were drawn in indian ink over high-contrast photographs of the gels. Kodak Microfile was used with a Wratten A25 red filter. Development was for 3 min. at 20° with Kodak formula D8 developer.

RESULTS AND DISCUSSION

Determination of haptoglobin types

The inherited serum-protein differences which have been demonstrated in normal individuals with the starch-gel electrophoretic method (Smithies & Walker, 1955) prove to be determined by differences in the haptoglobins of different individuals (Smithies & Walker, 1956). The haptoglobin type of a serum sample can be established by starch-gel electrophoresis either in the absence or in the presence of haemoglobin in the serum (Smithies, 1955*b*), since both the free haptoglobins and the complexes of haptoglobin and haemoglobin differ in their mobilities in starch gels in sera of the three haptoglobin types. An attempt to demonstrate differences in the haptoglobins of different individuals by filter-paper electrophoresis has been made. Fig. 1 shows photographs of the results obtained with typical sera of the three haptoglobin types (1-1, 2-1 and 2-2) in the absence of haemoglobin (1, 2 and 3) and in the presence (4, 5 and 6) of added haemoglobin (approx. 200 mg./100 ml.).

These results indicate that, in the absence of haemoglobin, sera of the three haptoglobin types are indistinguishable by filter-paper electrophoresis. In the presence of haemoglobin, however, changes occur in the filter-paper patterns which are characteristic for each type. Sera of haptoglobin type 1-1 in the presence of haemoglobin give two red zones. The slower of these is due to excess of haemoglobin, which migrates at the same rate as β-globulin, and the faster is due to the haemoglobin–haptoglobin complex. The complex migrates at essentially the same rate as α_2-globulin in sera of haptoglobin type 1-1 (see the arrow under pattern no. 4, Fig. 1). The position of this complex has been confirmed by two-dimensional electrophoresis.

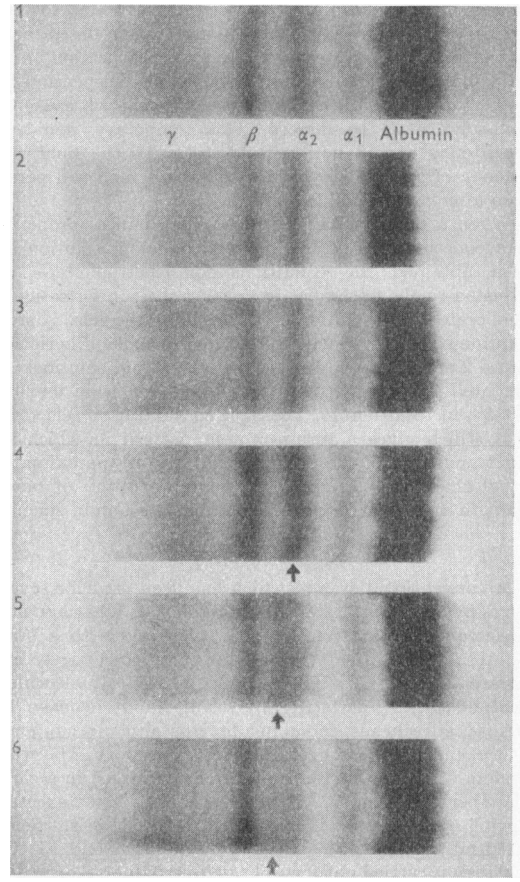


Fig. 1. Photographs of the filter-paper electrophoresis results obtained with typical sera of the three haptoglobin types (1-1, 2-1 and 2-2) in the absence of haemoglobin (strips 1, 2 and 3) and in the presence (strips 4, 5 and 6) of added haemoglobin (approx. 200 mg./100 ml.). The arrows under the photographs of strips 4, 5 and 6 indicate the positions of the haemoglobin–haptoglobin complexes.

In sera of haptoglobin type 2-1 the free haemoglobin migrates again at the same rate as β-globulin, but the haemoglobin–haptoglobin complexes in sera of this type migrate a little more slowly than α_2-globulin (see the arrow under pattern no. 5, Fig. 1) and give a characteristic appearance to the stained-protein patterns.

In sera of haptoglobin type 2-2 the haemoglobin–haptoglobin complexes migrate still more slowly, at a rate approximately half way between that of α_2-globulin and that of β-globulin. In some sera of haptoglobin type 2-2 sufficient haptoglobin is present for the complexes to be seen as a distinct zone between the α_2- and β-globulin zones.

Although these experiments demonstrate that filter-paper electrophoresis can be used to determine haptoglobin types, the reliability of the determinations is less than with starch-gel electrophoresis since the amount of haptoglobin present in individuals of the three types may vary considerably (see Plates 5 and 6). When the amount present is low a serum may be incorrectly typed unless the starch-gel method is used.

The relative rates of migration in the starch gels and on filter paper of free haptoglobin, free haemoglobin and their complexes provide confirmation of the hypothesis discussed by Smithies (1955*b*) that the additional resolution obtained in starch gels, compared with classical electrophoretic methods, is due to an additional effect of molecular size on rates of migration when starch gel is used as the supporting medium for electrophoresis. In sera of all three haptoglobin types the complexes of haemoglobin and haptoglobin migrate on filter paper at rates greater than free haemoglobin, although less than or equal to that of the haptoglobins not in the complex form. When the same sera are studied in starch gels the complexes always migrate more slowly than either free haemoglobin or the corresponding free haptoglobins. This indicates that the increase in molecular size which results from complex formation causes a decrease in the starch-gel mobilities to values less than those of the proteins not in a complex, although these same complexes migrate at intermediate mobilities on filter paper, and therefore probably in free solution also.

Correlation of the starch-gel and filter-paper electrophoresis components

The correlation of the components demonstrated by starch-gel electrophoresis with those demonstrated by filter-paper electrophoresis has been established by the method in which the proteins on serial sections of a filter-paper electrophoresis strip are compared with whole serum by a second electrophoresis in starch gels, and by the use of the two-dimensional system of electrophoresis. The correlation of the components established by these methods makes necessary a revision of the nomenclature of Smithies (1955*b*). In particular, it is proposed to cease using the terms post-β-globulin and αβ-proteins, which now prove to be misnomers. In the future it is suggested that, whenever possible, specific names be applied to the proteins, e.g. haptoglobins, copper-binding protein, etc. In most cases this is not yet possible. In the interim some method of description of starch-gel components, particularly those seen in the two-dimensional system, is necessary.

Description can be made primarily with reference to the classical five fractions of Tiselius. Proteins which migrate on filter paper between these fractions can still be referred to them; e.g. a protein migrating between the α_2- and β-globulin fractions can be referred to as an $\alpha_2\beta$-globulin. When any of the classical fractions comprises many components secondary identification can be made with reference to the starch-gel system, by assigning capital letters to the components of that fraction in order of their mobilities in the gels, e.g. α_2-globulin B would refer to a protein migrating in the α_2-globulin fraction on filter paper and having the second greatest starch-gel mobility of the α_2-globulins. Should, at a later date, any additional component be identified, e.g. between α_2-globulin A and α_2-globulin B, it could be given the next letter in the alphabet still unused for the respective fraction, e.g. α_2-globulin K, so that the letters used to describe already demonstrated components would not be changed on the discovery of new components.

Figs. 2 and 3 show the results of the two correlation methods applied to the serum of a normal individual of haptoglobin type 2-1. The small letters on the figures refer to the same starch-gel components, and the diagrams are arranged so that the directions of the migrations in the two figures have the same orientation. The legend of Fig. 3 indicates those zones which have been specifically identified with proteins isolated or described by other investigators, and summarizes the suggested method of describing the zones to which no specific names can yet be given.

A detailed discussion of these results and of the means whereby some of the zones have been identified follows.

Zone *a* is due to albumin which shows no sign of being resolved into more than one component by either electrophoretic system.

Three zones can be recognized in the α_1-globulin fraction (*b*, *c* and *d* in the figures). Zone *b* (originally referred to as 'pre-albumin$_2$' by Smithies, 1955*b*) has been identified as the acidic α_1-glycoprotein of Schmid (1950) and Weimer, Mehl & Winzler (1950)

by means of the combination of starch-gel electrophoretic and immunological methods described by Poulik (1956). Specific antiserum against the acidic α_1-glycoprotein was kindly given to the authors by Dr Morris Goodman, Detroit Institute for Cancer Research. The acidic α_1-glycoprotein which was used as the antigen to produce this antiserum in chickens (Goodman *et al.* 1957) was prepared by a modification of the methods of Schmid (1950) and Weimer *et al.* (1950). Zones *c* and *d* are not yet identified and will therefore be designated as α_1-globulin B and α_1-globulin C respectively. α_1-Globulin B is the quantitatively major component of the α_1-globulin fraction. In one-dimensional starch gels α_1-globulin B is unresolved from albumin.

Zone *e* ($\alpha_1\alpha_2$-globulin A) is a component which on filter paper migrates at a rate between that of α_1-globulin and α_2-globulin. In one-dimensional gels $\alpha_1\alpha_2$-globulin A is unresolved from the main β-globulin fraction (zone *s* in Figs. 2 and 3).

The great complexity of the α_2-globulin fraction is shown by filter-paper section no. 8 (Fig. 2), and by the two-dimensional experiment. At least ten components can be recognized. Zones *f* and *g* (α_2-globulins A and B) have not been identified (they were previously referred to as 'post-albumins'). Zone *h* is a haptoglobin present in only small amounts in sera of haptoglobin type 2-1. This haptoglobin is present in much greater amounts in sera of haptoglobin type 1-1, in which it is the only haptoglobin present. Thus the heterozygote, Hp^2/Hp^1 (Smithies & Walker, 1956), has small amounts of the haptoglobin which characterizes the homozygote, Hp^1/Hp^1. Zone *i* is not identified and may be designated α_2-globulin D. It is not a haptoglobin and is part of the starch-gel fraction originally referred to as 'Fα_2-globulin.' Zones *j*, *k*, *l*, *m* and *n* are the characteristic haptoglobins of sera of haptoglobin type 2-1. These should not be referred to as α_2-globulins E, F, G, H and I, since they are haptoglobin type-specific and such nomenclature would lead to confusion with the similar, but not identical, haptoglobins of type 2-2 sera. They may be designated type 2-1 haptoglobins. The fastest-migrating type 2-1 haptoglobin (zone *j*) is not readily resolved in one-dimensional gels from zone *p* ($\alpha_2\beta$-globulin A); it is present in all sera of haptoglobin type 2-1, but not in sera of types 1-1 or 2-2. The presence of this protein is the cause of the observation of Smithies (1955*b*) that the 'post-β-globulins' in type 2-1 sera (now known frequently to comprise two components) are usually less well resolved from β-globulin than in type 2-2 sera.

Zone *o* is the most slowly migrating α_2-globulin (Sα_2-globulin). The irregular zone immediately adjacent to *o* is due to traces of Sα_2-globulin which are temporarily adsorbed on the filter paper, and is seen only when the serum is inserted into gels on

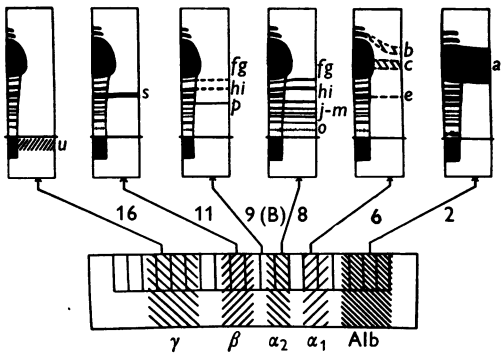


Fig. 2. A diagrammatic representation of the results of electrophoresis in starch gels of the proteins in serial sections taken from a filter-paper electrophoresis strip (serum of haptoglobin type 2-1) compared with the proteins of whole serum. On the right-hand side of each gel are seen the proteins present in one of the serial sections. On the left-hand side are the proteins of the whole serum. The numbers on the figure are the serial numbers of the sections. The key to the letters on the figure is given in the legend of Fig. 3.

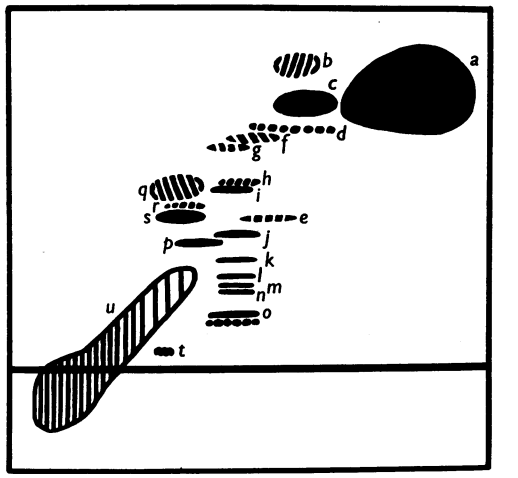


Fig. 3. A diagrammatic representation of a two-dimensional electrophoresis experiment on serum of haptoglobin type 2-1. The filter-paper strip obtained from the first electrophoresis was inserted into the gel in the position shown by the solid line across the figure. The second (starch-gel) electrophoresis was carried out at right angles to this line. Key to lettering (applies also to Figs. 2 and 4): *a*, albumin; *b*, acidic α_1-glycoprotein; *c*, α_1-globulin B; *d*, α_1-globulin C; *e*, $\alpha_1\alpha_2$-globulin A; *f*, α_2-globulin A; *g*, α_2-globulin B; *h*, type 1-1 haptoglobin; *i*, α_2-globulin D; *j*, *k*, *l*, *m* and *n*, type 2-1 specific haptoglobins; *o*, Sα_2-globulin; *p*, $\alpha_2\beta$-globulin A; *q*, β-globulin A; *r*, β-globulin B; *s*, β-globulin C; *t*, high-molecular-weight β-lipoprotein; *u*, γ-globulins.

filter paper. Since $S\alpha_2$-globulin is the most slowly migrating α_2-globulin in the gels it would be expected to be the fraction of greatest molecular size. Confirmation of this expectation has been obtained. Dr Henry G. Kunkel of the Rockefeller Institute, New York, kindly supplied the authors with a highly purified α_2-globulin (a glycoprotein) of sedimentation coefficient 19s prepared by electrophoretic and ultracentrifugal methods (Wallenius, Trautman, Kunkel & Franklin, 1957). This material was shown by electrophoresis in the gels to correspond to $S\alpha_2$-globulin. When serum is stored the $S\alpha_2$-globulin shows an increase in its starch-gel mobility, which suggests that it is labile. An antiserum against the heat-labile glycoprotein of Brown, Baker, Peterkovsky & Kaufman (1954) was prepared by the injection into rabbits of material kindly supplied by Dr Ray K. Brown, New York State Department of Health, New York. This antiserum reacted specifically with $S\alpha_2$-globulin. It is thus probable that $S\alpha_2$-globulin, the α_2-glycoprotein (19s) and heat-labile glycoprotein are identical.

Zone *p* is particularly well shown on the *bottom* of the gel corresponding to the filter-paper section labelled 9 (B). On the bottom of the gel the proteins from the edge closer to β-globulin of this filter-paper section will be seen. (All other drawings in Fig. 2 depict the proteins in the middle of the gels.) This result clearly indicates that this protein migrates between α_2-globulin and β-globulin on filter paper, as is confirmed by the two-dimensional experiment. Consequently the protein can suitably be referred to as $\alpha_2\beta$-globulin A.

Four zones (*q*, *r*, *s* and *t*) can be seen in the β-globulin fraction in the two-dimensional experiment illustrated. Zones *q*, *r* and *s* have not been identified and may be designated β-globulins A, B and C. β-Globulin C (see filter-paper section no. 11 in Fig. 2) is the quantitatively predominant β-globulin in the majority of sera investigated at the present time. However, in some individuals an additional protein (β-globulin D) is seen which migrates slightly less rapidly than β-globulin C, both in the starch gels and on filter paper, and is present in approximately the same amount as β-globulin C. This β-globulin will be discussed in more detail in a later paper. Zone *t* stains with lipid stains. In one-dimensional gels the chief lipid-staining component of serum migrates at this rate (see also Silberman, 1957). Zone *t* is thus probably β-lipoprotein of high molecular weight.

The characteristic appearance of the γ-globulin zone (*u*) in the two-dimensional gels suggests that there is a distribution of protein components in this serum fraction with an essentially continuous range of mobilities. This observation is in accordance with the electrophoretic studies of Müller-Eberhard & Kunkel (1956) on purified Cohn fraction II γ-globulin.

The nature of 'pre-albumin$_1$' (old nomenclature) relative to the classical methods has been determined by a separate experiment in which the filter-paper electrophoresis was carried out for only a short time (6 hr.) so that this component had not diffused to an undetectable concentration. It migrates faster than albumin on filter paper as well as in the gels and is thus, on the revised nomenclature, termed pre-albumin A. It is probably equivalent to the pre-albumin described by Hoch & Chanutin (1953) and Schutze, Schönenberger & Schwick (1956).

A two-dimensional experiment performed on a normal individual of haptoglobin type 1-1 is illustrated in Fig. 4, for comparison with the results on the serum of haptoglobin type 2-1. The characteristic haptoglobin (zone *h*) of this type 1-1 serum is present in considerable quantity. No other haptoglobins are present. In other respects this serum is equivalent to the type 2-1 serum.

No evidence is seen in the two-dimensional experiments of any protein components which fail to enter the gel, except where the extended γ-globulin zone intersects the insertion slit and where the protein would not be expected to migrate away from the filter paper. This suggests that proteins of a very wide range of molecular sizes can enter the gels. However, as noted above, the starch-gel resolutions appear to result from a greater effect of molecular size on mobilities than is the case in free solution. These two observations may be understood if the gel is regarded as a network of polysaccharide chains which are in continual thermal movement. There will be a continually fluctuating

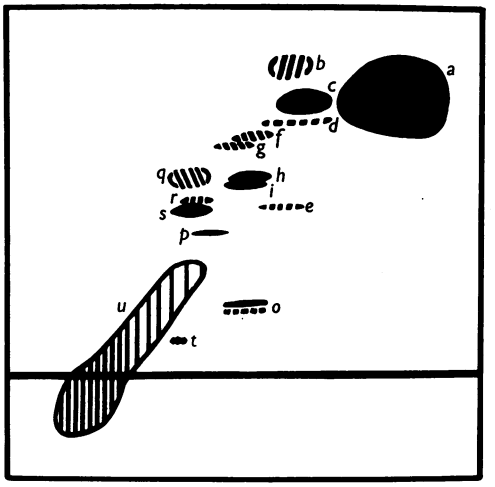


Fig. 4. A two-dimensional experiment on serum of haptoglobin type 1-1. The key to the letters on the figure is given in the legend of Fig. 3.

range of 'pore sizes' at a given position in the gel. If a large 'pore' is required, to permit the passage of a protein of large molecular size, a longer time will on the average elapse before such a 'pore' is available than for a small 'pore'. Thus proteins of large molecular size will be hindered in their migration but will not be prevented from entering the gels, unless additional factors, such as solubility in the dilute buffer solution or great asymmetry (e.g. fibrinogen), are involved.

Abnormal sera

A small number of abnormal sera have been studied by the starch-gel and filter-paper methods in order to confirm the correlations of the starch-gel components with the classical five components which have been established by the studies on normal sera, and to illustrat the possibilities of the starch-gel method for the study of abnormal sera. Serum samples in which, where possible, only one of the five main serum-protein fractions was increased were selected for this study. These sera were obtained from patients in the teaching hospitals of the University of Toronto. The authors have not attempted to speculate on the interpretation of the results in terms of the pathological states of the patients since in many instances only one specimen was studied.

Plates 5 and 6 illustrate photographically the results obtained in the starch gels. The apparent dye intensities in the different gels are comparable since standard photographic conditions of exposure and development were used. The filter-paper results are illustrated by photographs of the tracings obtained on the cathode-ray tube of an electronic computer for scanning filter-paper strips (Pinteric & Poulik, 1954; Poulik & Pinteric, 1955). The vertical co-ordinate in these tracings corresponds to the concentration of dye bound to protein, the horizontal co-ordinate to distance along the filter-paper strips.

A summary of the chief points of interest in each serum sample follows. The results on serum from a normal individual (see Plate 5) are included for comparison with the abnormal sera.

Serum 1. Male, 22 years. Normal individual. Haptoglobin type 2-2. Serum from this same person was used as a typical example of haptoglobin type 2-2 serum in the experiments illustrated in Fig. 1.

Serum 2. Male, 56 years. Carcinoma of the lung. Haptoglobin type 2-2. Total protein not available. The increases in both the α_1- and α_2-globulin fractions demonstrated by the filter-paper method in this serum are seen in the starch-gel result. The acidic α_1-glycoprotein, the type 2-2 haptoglobins and the $S\alpha_2$-globulin are markedly increased. In all sera so far examined in which filter-paper electrophoresis shows an increase in the α_1-globulin fraction the starch-gel method shows that the acidic α_1-glycoprotein ('pre-albumin$_2$') is increased.

Serum 3. Female, 66 years. Nephrotic syndrome. Haptoglobin type 2-2. Total protein 3·5 g./100 ml. The filter-paper experiment indicates that the α_2-globulin fraction forms the major protein constituent of this serum. The starch-gel experiment shows that the larger molecular size α_2-globulins ($S\alpha_2$-globulin and the type 2-2 haptoglobins) have been retained in the circulation, as might reasonably be expected in this condition. Acidic α_1-glycoprotein is present in greater amounts than in normal sera.

Serum 4. Female, 54 years. Multiple myeloma. Haptoglobin type could not be determined. Total protein 8·8 g./100 ml. A grossly abnormal β-globulin is present in the serum of this individual by both methods. The amount of γ-globulin is considerably reduced.

The following results (see Plate 6) are included to demonstrate the differences in the γ-globulins of a number of abnormal sera which can be shown unambiguously with the starch-gel electrophoretic method. The differences are much less clearly defined in the filter-paper experiments.

Serum 5. Male, 76 years. Arteriosclerotic heart disease with congestive heart failure, carcinoma of

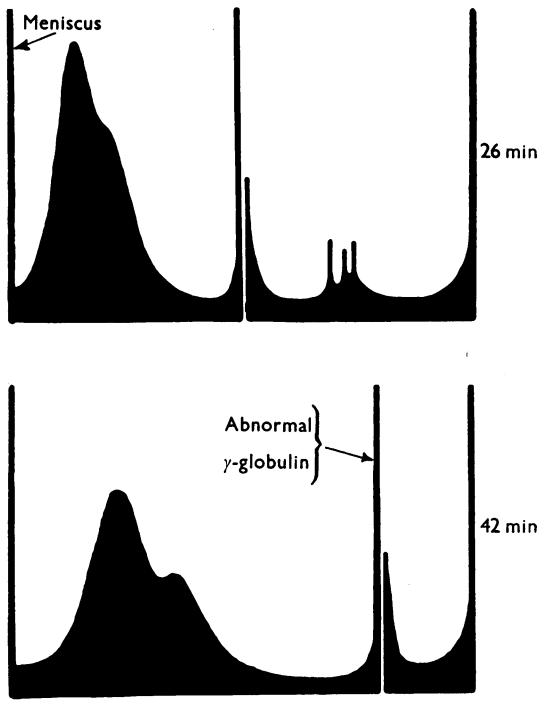
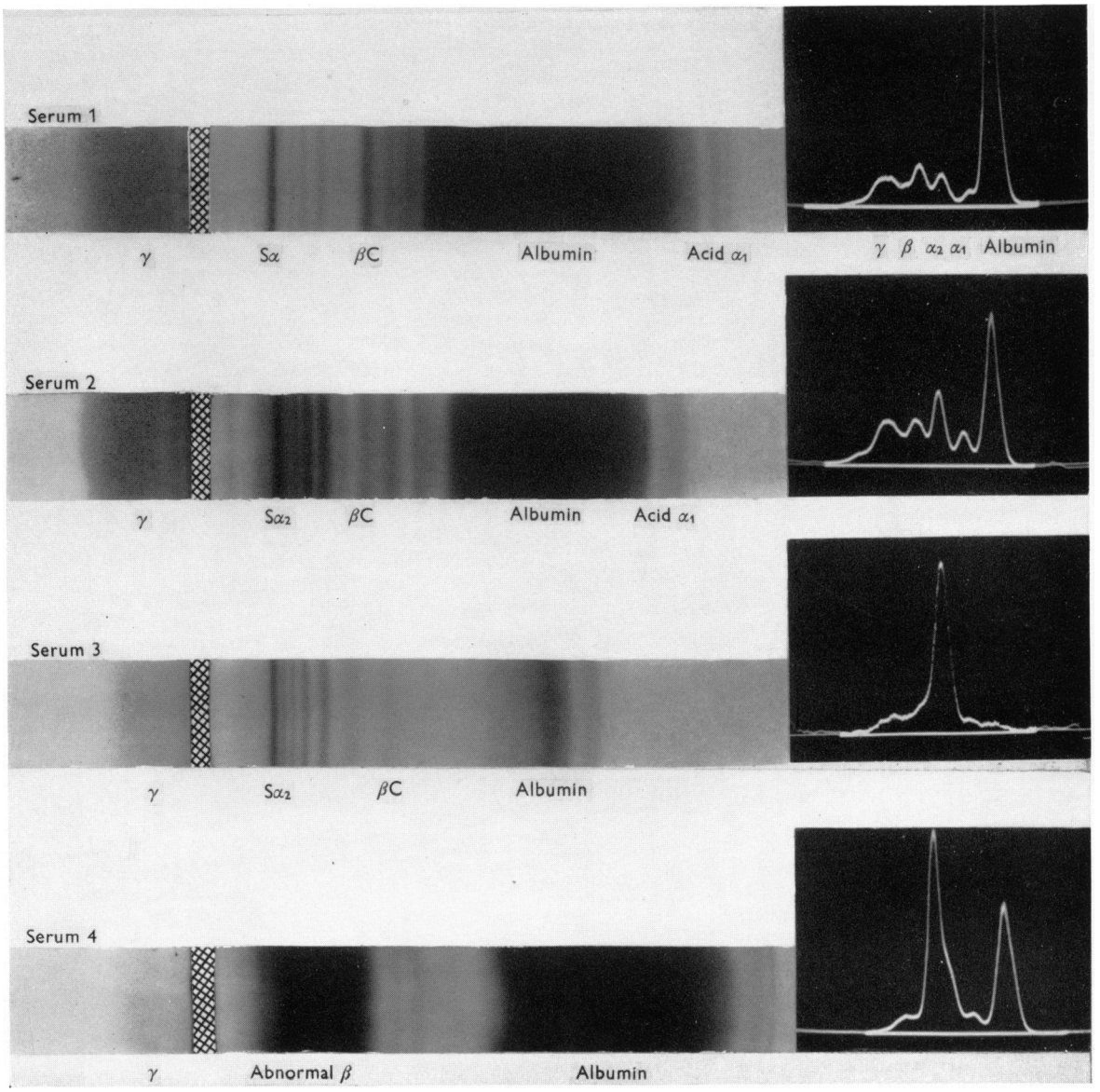


Fig. 5. Ultracentrifuge diagrams of serum 5 (see Plate 6) diluted [1 vol. of serum plus 3 vol. of 1% (w/v) NaCl]. Spinco ultracentrifuge, speed 59 780 rev./min. Temp. 25·6°. Upper diagram: 26 min. after reaching 59 780 rev./min. Lower diagram: 42 min. after reaching 59 780 rev./min.



Plates 5 and 6. A comparison of the results of starch-gel and filter-paper electrophoresis experiments on serum samples from eight individuals. The starch-gel experiments are illustrated by photographs of the stained gels. The filter-paper experiments are illustrated by photographs of the tracings on the cathode-ray tube of an electronic computer for scanning stained filter-paper electrophoresis strips. Serum 1 is normal. Sera 2–8 show abnormal features.

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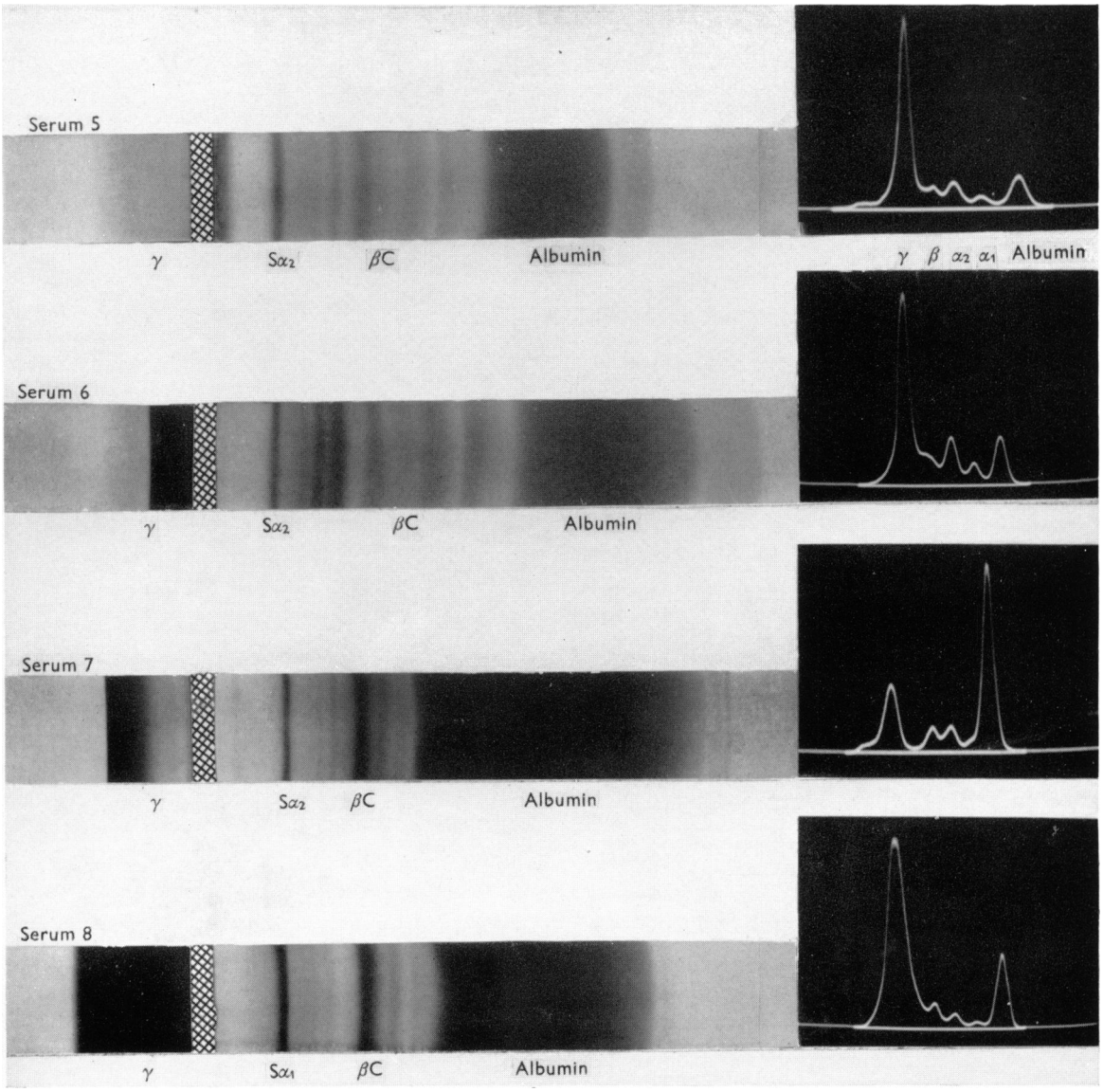

For legend see Plate 5.

the prostate (confirmed *post mortem*). Haptoglobin type 2-1. Total protein 6·8 g./100 ml. The abnormal γ-globulin present in considerable quantities in this serum largely failed to enter the starch gels and formed a layer on the positive side of the slot used for sample insertion. The presence of considerable amounts of an exceptionally large molecular size or highly asymmetric protein was inferred from this behaviour. Examination of the serum in the ultracentrifuge showed that the abnormal protein was of high molecular weight and suggested that it was highly asymmetric (see Fig. 5). This protein thus probably fails to enter the gels because of the same factors as prevent fibrinogen from entering the gels.

Serum 6. Female, 55 years. Multiple myeloma (confirmed *post mortem*). Haptoglobin type 2-1. Total protein 8·3 g./100 ml. Blood from this individual clotted in about 1 min. with very little clot retraction on standing. Despite this suggestion of macroglobulinaemia the abnormal γ-globulin enters the gel and shows as a very darkly staining area in the gel on the negative side of the insertion slot. The behaviour in the starch gel of the γ-globulin from this patient is quite different from that seen with the serum sample 5, although the filter-paper results are very similar.

Serum 7. Female, 51 years. Mother of juvenile diabetic. Serum from this supposedly normal individual was obtained for genetic studies. The starch-gel result suggested multiple myeloma, and further examination of this woman showed clinical signs of this condition which had previously been overlooked. Haptoglobin type 2-1. Total protein was not available. Both the filter-paper and starch-gel results show an abnormal distribution of proteins in the γ-globulin fraction.

Serum 8. Female, 13 years. Uncertain diagnosis, possibly a lupus erythematosis type of condition. Haptoglobin type 2-2. Total protein 12 g./100 ml. A marked generalized increase in the γ-globulin is seen in this serum by both methods.

The studies summarized in the Plates show that changes in the proteins of abnormal sera which can be demonstrated to occur in a given serum-protein fraction by filter-paper electrophoresis are also found to occur in the corresponding fractions by starch-gel electrophoresis. The considerable increase in information which can be obtained by the use of the starch-gel method is apparent.

The series of abnormal sera presented in the Plates also indicates the need for care in the identification of the starch-gel zones in grossly abnormal sera. In such sera the rates of migration of the proteins in the one-dimensional gels may differ slightly from those normally seen, since the apparent mobilities of the different proteins are to some extent interdependent. However, the starch-gel components in abnormal sera may still be identified relative to the normal components by comparing, in a single starch gel, the abnormal serum with a normal serum of the same haptoglobin type.

SUMMARY

1. The results obtained by the electrophoresis of the proteins of normal and abnormal sera by the starch-gel and filter-paper methods are compared. The considerable increase in information given by the starch-gel method is apparent.

2. The components demonstrated in the gels are correlated with those seen on filter paper, by the use of a suitable combination of the two methods.

3. A two-dimensional electrophoretic system (the first dimension on filter paper, the second at right angles in starch gel) is described in detail. More than 20 serum-protein components can be demonstrated with this system.

4. A nomenclature suitable for describing the components demonstrated by the starch-gel methods is proposed.

5. Several of the components have been identified with specific proteins previously isolated or described by other workers.

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