

An Improved Procedure for Starch-gel Electrophoresis: Further Variations in the Serum Proteins of Normal Individuals

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Experience with the method of zone electrophoresis in starch gels (Smithies, 1955) suggests that the resolution of protein components is lessened by the use of supporting substances (e.g. filter paper or starch grains) to prevent electro-decantation from occurring during the entry of migrating proteins into the gels. The present paper describes a simple procedure which enables the sample to be inserted into the gel without any supporting substance. Electro-decantation is prevented by carrying out the electrophoresis with the gel in a vertical position. The resolution of serum proteins obtainable in the gels is considerably improved, and the difficulties of obtaining reproducible results are reduced. Evidence is obtained with the new technique that genetic factors are concerned in the control of the 'post-albumins' of different persons.

EXPERIMENTAL METHODS

Apparatus. Figs. 1, 2 and 4 show the design of the apparatus most frequently used by the author for vertical starch-gel electrophoresis. The size of gel illustrated enables eight samples to be compared at once. However, the width of the gel can be varied according to need (e.g. for a single sample a gel 2 cm. wide has been used) and the gel can be varied in length. The volume of sample inserted into the gels can be changed by replacing the removable piece of plastic used to form the slots (Fig. 2B) by a piece made from plastic sheet of a different thickness.

Procedure. The cover for the gel tray (Fig. 2) is lightly smeared with mineral oil (except for the piece of plastic used to form the multiple slot) and is put in an oven at approx. 70°. The tray (Fig. 1) is placed horizontally with the end plates secured in position. The starch solution is prepared (see Smithies, 1955, and Poulik & Smithies, 1958)

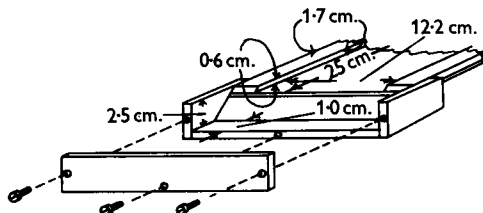


Fig. 1. 'Exploded' view of one end of the plastic tray with removable end plates used for vertical starch-gel electrophoresis. Only important dimensions are given.

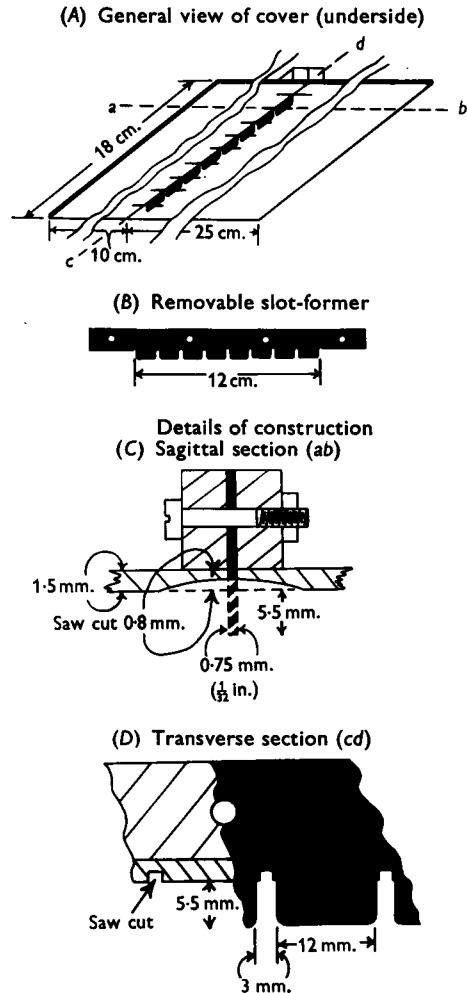


Fig. 2. General view (A), and constructional details (C and D), of the plastic cover used to form the eight sample slots during the casting of the gel. The removable slot-former (B), shown throughout the figure in black, is made from linen-based phenol-formaldehyde resin ($\frac{3}{8}$ in. sheet); other plastic parts are made from Plexiglas. Note the saw cuts made with a circular saw at right angles to the slot-former. These cuts are made after the cover is assembled, and cause small ridges of gel to be formed between adjacent sample slots.

and is poured into the tray. An excess of gel should be used (500 ml. for the tray illustrated). The hot cover is then carefully lowered into position without trapping air bubbles, and weights are put on the corners of the cover until the gel is cool.

The cover is removed carefully so as not to damage the slots in the gel. The removal is facilitated if the cover is first freed from the gel up to the region of the slots, and is then gently prised off one side with a spatula opposite the slots. The samples (approx. 0.05 ml.) are run into the slots with Pasteur pipettes. Usually the two outer samples are duplicated as the zones tend to be curved at the edges of the gel. The small ridges of gel between the sample slots, which are formed by the circular-saw cuts in the cover (see Fig. 2C, D), prevent inter-transfer of samples between adjacent slots.

Petroleum jelly at approx. 45° is poured directly over the samples in the slots to seal them in position, and the remainder of the exposed surface of the gel is then covered with additional melted petroleum jelly. The end plates of the gel tray are removed, and the gel is assembled in a vertical position, as shown in Fig. 3, so that the albumin will migrate downwards. The sample slots are carefully levelled in both planes. This step is critical, and a spirit level should be used to check the levels otherwise the samples will not enter the gel uniformly in all planes.

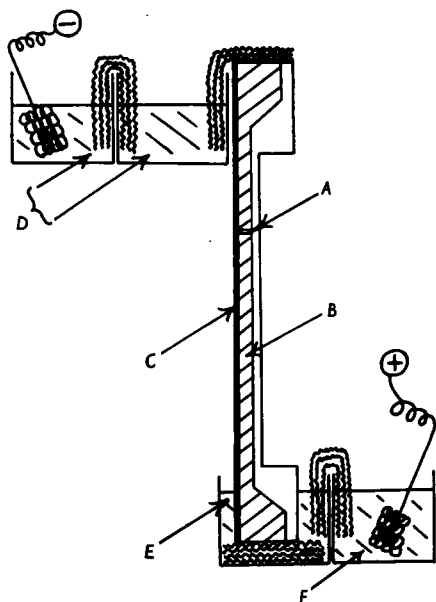


Fig. 3. Experimental arrangement for vertical starch-gel electrophoresis. A, position of sample slots; B, gel; C, petroleum jelly seal; D, trays containing bridge solution (0.3M-boric acid, 0.06M-NaOH). The negative reversible Ag/AgCl electrode is in the outer tray. E, Tray containing bridge solution with approx. 10 thicknesses of filter paper at the bottom, on which the end of the gel rests. F, Tray containing concentrated NaCl soln. (approx. 10%, w/v) for the positive reversible Ag/AgCl electrode. The zig-zag lines in the figure show the positions of thick filter-paper bridges.

The same type of result can be obtained with the gel and electrodes arranged so that migration is upwards, but some irregularity in the zones may then occur because of density instability in the sample slots. On the other hand, the gel slots need not be so accurately levelled when migration is upwards. Nevertheless the overall results favour migration downwards.

A voltage gradient of 5 v/cm. (i.e. 135 v across the gel here described) applied for 16–20 hr. has been used by the author for work on serum proteins with borate buffer gels prepared from commercially available starch (Starch-Hydrolysed, Connaught Medical Research Laboratories, Toronto). The pre-albumins and some albumin usually migrate out of the gel with the longer times, but the gels can be lengthened if this is undesirable.

At the end of the electrophoresis the petroleum jelly is removed from the surface of the gel. The gel is trimmed square at the ends, and is turned out on to the slicing tray illustrated in Fig. 4. The gel is placed upper surface downwards with the insertion slots away from the 6 mm. thick 'stopping block' of the slicing tray. Air bubbles are carefully pressed out from between the gel and the tray. Slicing is carried out with a dermatome-knife blade in one smooth motion from the γ -globulin end of the gel to the albumin end. The cut gel surfaces are stained in the usual way with Amido-Black 10 B (Smithies, 1955).

Photography. The photograph was taken on Kodak 35 mm. Microfile with tungsten-lamp illumination and a Wratten A (red) filter. Development was carried out with Kodak formula D23 developer for 12 min. at 20°.

RESULTS AND DISCUSSION

Plate 6 shows the result obtained with the vertical starch-gel technique with sera from six healthy persons. The section of gel from the sample slots to the albumin is included in the photograph. This result illustrates the type of resolution obtainable with the improved technique. The particular sera

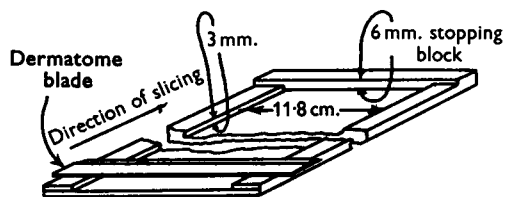
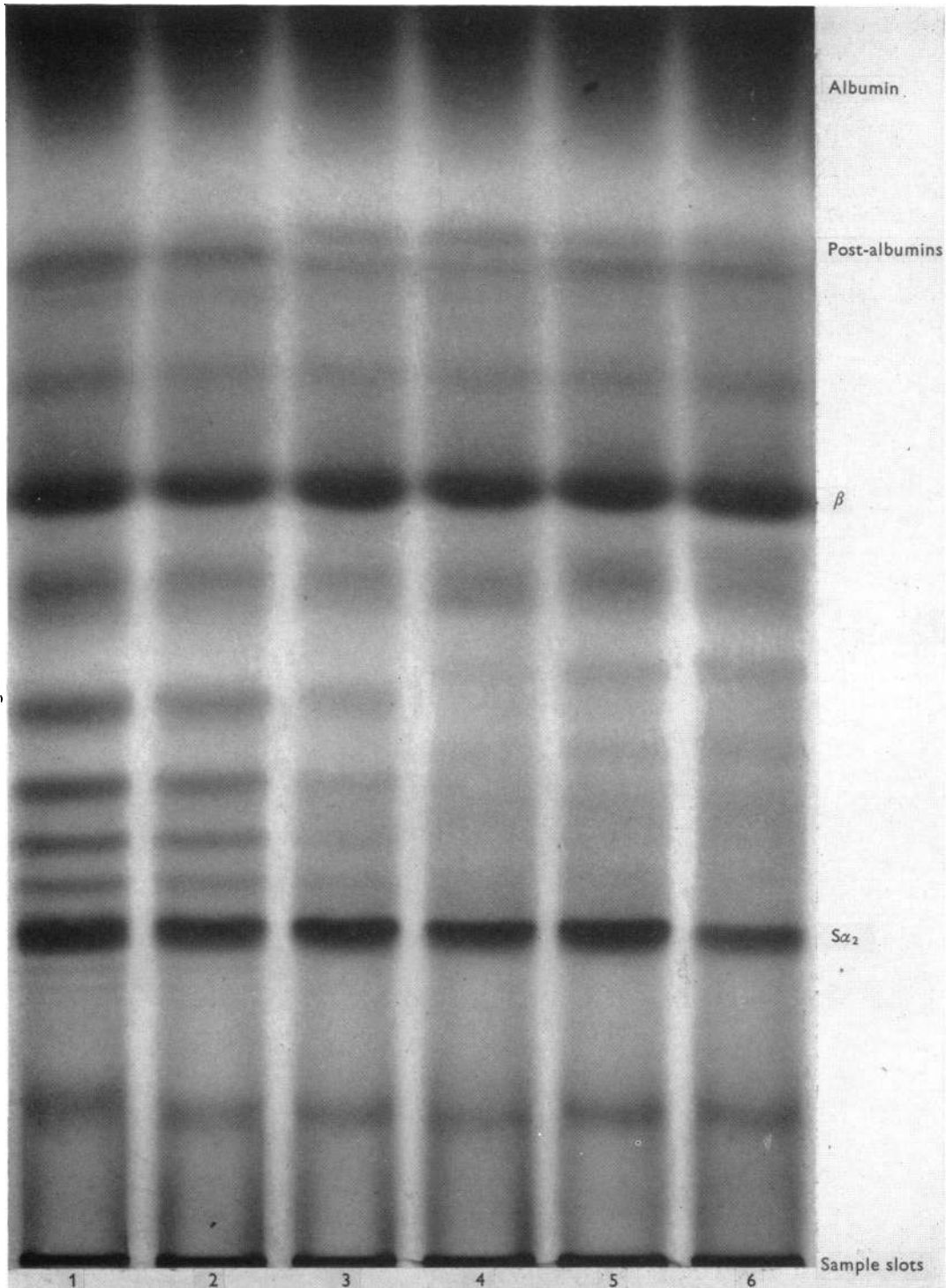


Fig. 4. Slicing tray on which the gel is sliced with a dermatome-knife blade. Only important dimensions are given.

EXPLANATION OF PLATE 6

A photograph of the result obtained by vertical starch-gel electrophoresis (approx. 19 hr. at 5 v/cm.) with serum samples from six healthy individuals. Only the section of gel from the sample slots to the albumin is included in the photograph. Samples 1 and 2 are from female identical twins, 45 years old. Samples 3–5 are from 9-year-old female non-identical quadruplets and sample 6 is from their mother.



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selected for this experiment point out the potential of the technique in further studies of the genetic control of serum proteins, particularly in the region of the post-albumins.

The two samples (1 and 2) on the left-hand side of the gel were taken from a pair of female twins, aged 45 years (1 is unmarried; 2 is the mother of four children). The haptoglobin and β -globulin types (Smithies & Walker, 1956; Smithies, 1958) of these individuals are the same. The resolution of the type 2-2 haptoglobins in the sera of the twins is very satisfactory (as many as 12 haptoglobin zones can be distinguished in the original photograph), and the high-molecular-weight β -lipoprotein shows as a clear zone between the α_2 -globulin and the sample slot. Many of these haptoglobin zones and the β -lipoprotein were not reproducibly demonstrated by the earlier starch-gel technique. The greatest improvement in resolution is in the post-albumin region. The sera from the twins show post-albumins which are essentially indistinguishable, as indeed are all the protein zones which can be detected in these two serum samples. The twins are known to be identical (monozygotic) on the basis of their red-cell blood groups and other tests for their zygotic state.

Serum samples 3-5 are from three female quadruplets, aged approx. 9 years. (A sample from the remaining quadruplet was not available.) The three quadruplets here concerned are known, from their red-cell blood groups, to be non-identical. The haptoglobin types of the first two quadruplets (samples 3 and 4) differ and are Hp2-2 and Hp2-1 respectively. On the other hand, the post-albumins of these two children are indistinguishable, although they differ from those of the adult twins (samples 1 and 2). The haptoglobin type of the third quadruplet (sample 5) is the same, Hp2-1, as that of one of her sisters (sample 4), but the post-albumins of this third quadruplet are clearly different from those of either of her sisters or of the twins.

Serum sample 6 is from the mother of the

quadruplets. Her haptoglobin type is 2-1, and her post-albumins are essentially indistinguishable from those of one of her daughters (sample 5).

In conclusion, the results with these six serum samples illustrate the improved resolving power of the vertical starch-gel electrophoresis technique, and suggest strongly that genetic factors are involved in the control of the post-albumins of normal individuals. Thus the (identical) twins, despite their very different histories, have essentially indistinguishable post-albumins, yet two of the (non-identical) quadruplets show differences. The variability in this region appears to be complex, and other 'post-albumin types' have been observed in addition to the three illustrated in the Plate. Samples taken almost a year apart from one individual (whose post-albumins are very characteristic) gave the same pattern, a result which would be expected if genetic factors are concerned in the control of the post-albumins. Further work is in progress to characterize these differences more fully and to investigate their genetic control.

SUMMARY

1. An improved technique is described for starch-gel electrophoresis in a vertical direction which permits the sample to be introduced into the gel without the use of any supporting substance.
2. The resolving power and reproducibility of the method are thereby much improved.
3. Evidence is presented which suggests that genetic factors are involved in the variations, in different individuals, of the serum proteins which migrate immediately behind albumin.

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