A MODIFIED DISC ELECTROPHORETIC METHOD FOR ANIMAL BLOOD SERUM PROTEINS*

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Recent advances in zone electrophoretic methods have provided a fund of knowledge on protein components of animal and vegetable tissues, hitherto unavailable. In particular, the disc electrophoretic method developed by ORNSTEIN AND DAVIS[1] has routinely demonstrated over 20 components with human serum as compared with 5 by conventional Tiselius electrophoresis. In connection with rat experiments in progress in our laboratory, it was found necessary to determine electrophoretically all the serum protein components using as small a quantity of blood as possible.

In the standard disc electrophoretic procedure, the sample is polymerized in a large pore size acrylamide gel. Frequently the polymerization of this sample gel is inhibited and could give rise to erroneous results. In the present communication, micro quantities of blood were drawn from the animals and their serum electrophoretic pattern determined using a modified disc electrophoretic procedure which avoids use of sample gel as the anti-convection medium. In order to indicate the versatility of the method, the technique has been extended to four other small animals (chick, mouse, guinea pig and rabbit) and, to our knowledge, disc electrophoretic patterns of their sera have not been previously reported.

MATERIALS AND METHODS

Collection of blood and serum preparation

Micro quantities of blood were collected in hematocrit capillary tubes from either animals or human beings making use of a lancet prick. The tail was pricked in the case of the rat and the mouse, the ear in the case of the rabbit and guinea pig and the pectoral region in the case of the chick. The tubes were sealed with citrocaps and the blood immediately centrifuged for 15 min in an International clinical centrifuge. The serum was separated from the sedimented material by cutting the capillary tube and the serum quantitatively transferred to a glass vial and "solution B-sucrose" solution was added (0.23 ml per cm of serum in the capillary tube as measured with a cm scale).

Calibration of the capillary tubes

The tubes used were those distributed by Aloe Scientific Company. Several tubes

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chosen at random were calibrated using mercury. The volume per cm of the capillary tube was 7.4 μl $\pm$ 0.3 where 0.3 is the sample standard deviation.

Electrophoretic procedure

The procedure was the same as that described by ORNSTEIN AND DAVIS[1], except for the replacement of "solution B-sucrose" solution instead of the upper gel solution as the anti-convection medium. The spacer gel was gently overlaid with the sample solution and inserted into the upper reservoir with care. The current used was usually 2.5 mA/tube and the time of run was usually 35 min for animal sera and 55 min for human sera.

"Solution B-sucrose" solution

This solution was prepared by mixing 10 ml of solution B[1], 30 ml of distilled water and 40 ml of 20 % (w/v) sucrose solution.

RESULTS AND DISCUSSION

Although there are electrophoretic methods which make use of small quantities of serum, there are no suitable methods available which describe use of micro quantities of blood as the starting material. Generally a relatively large quantity of the blood is obtained from an animal and allowed to clot for a period of 2 to 4 h and the serum obtained by centrifugation. In all the electrophoretic runs described below, only 7 to 40 μl of blood were collected in capillary tubes and the serum obtained by centrifuging immediately for 15 min as described earlier. BARRETO[2] has recently developed a method for the preparation of lambda quantities of serum for paper electrophoresis. However, his method uses larger quantities of blood, may involve heat denaturation of the labile proteins, and requires transfer of serum from the capillary tube to a pipette for quantitative measurement.

The results obtained with human sera are shown in Figs. 1–4, and those for the various animal sera in Figs. 5 and 6. Figs. 5 and 6 represent normal blood serum electrophoretic patterns of five common laboratory animals as follows: A 227-1 and 2 albino rat; A 237-1 and 2 chick (crossbreed); A 285-5 and 6 mouse; 291-1 and 2 guinea pig; 291-5 and 6 rabbit. All samples were run in duplicate and the reproducibility will be apparent from the photographs. A tentative identification of the bands for human serum has been made by ORNSTEIN AND DAVIS[1]. It was suggested that the leading faint band was the prealbumin and was followed by the heavy albumin band, the three postalbumins, the transferrin, the region of haptoglobins and "7 S" γ-globulins, slow α_2-macroglobulin and β-lipoprotein. In the case of starch gel, the bands have been identified by SMITHIES for human serum[3] and by BEATON *et al.* for rat serum[4]. On this basis, the following tentative identification is suggested for rat serum protein bands. A faint prealbumin was followed by the albumin band, three to four postalbumins, one or two transferrins, fast α_1- and α_2-globulins and slow α_1- and α_2-globulins, and finally a faint β-lipoprotein band. A total of 14 bands was seen in the gels and can be compared with a similar number obtained by BEATON *et al.* using two-dimensional filter paper–starch gel electrophoresis. Possibly the identification suggested here may also be extended to the other animal serum proteins. The number of components seen in the gels themselves under the present experimental conditions

for the various sera were: Human 23; rat 14; chick 11; mice 11; guinea pig 11; rabbit 11. In the case of mice, using starch gel electrophoresis CONS AND GLASS[5] report that 11 bands were observed. The quantity of serum used by them was about 16 times the quantity used in the present experiments.

In order to illustrate the reproducibility of the present method, human blood serum from a single individual was used in one experiment (Fig. 1). The time of run was 7 min shorter than normal and hence the fine haptoglobin and γ-globulin bands were not resolved. However, the reproducibility of the method can be inferred from the albumin and transferrin bands.

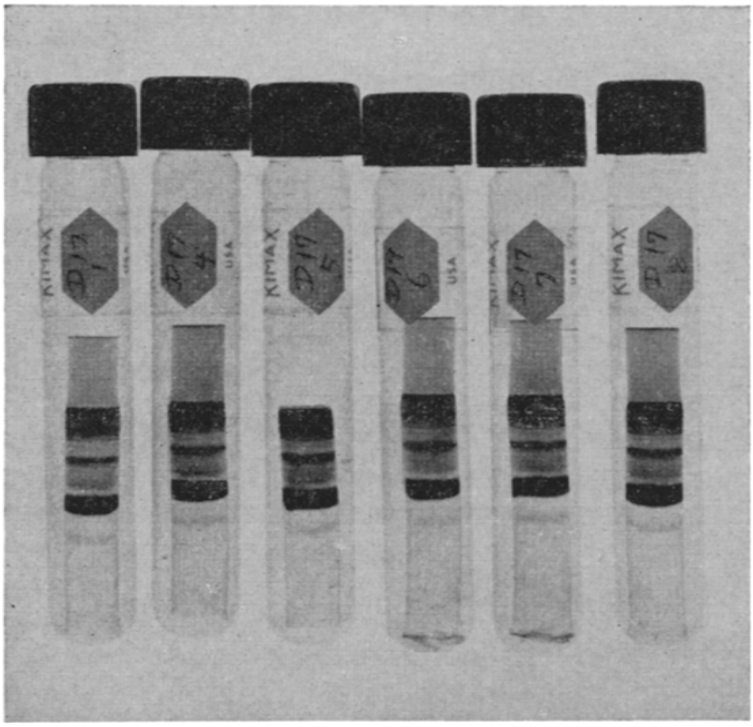

Fig. 1. Human blood serum electrophoretic patterns. D 17-1, 4, 5, 6, 7, 8 human serum, identical samples, using "solution B-sucrose", 3.9 μl, 48 min at 2.5 mA.

In our earlier experiments conducted according to the procedure of ORNSTEIN AND DAVIS where the sample was polymerized in the sample gel, we encountered problems of improper sample gel polymerization, intense background staining in the γ-globulin region and a certain amount of protein precipitation at the sample gel–spacer gel interface. Fig. 2 depicts photographs of runs obtained with identical amounts of human serum taken from a single individual using standard disc electrophoretic conditions but in which the sample gel failed to polymerize completely. It was evident that there was poor reproducibility under these conditions. Although the reason why these sample gels did not polymerize was not known, the following factors are known to inhibit polymerization: slight hemolysis, protein concentration, aging of upper gel solution, traces of acids and increased amounts of specific proteins such as globulins.

Improper sample gel polymerization appeared to be a common problem for many laboratories and was referred to at a recent conference on gel electrophoresis[6]. Further-

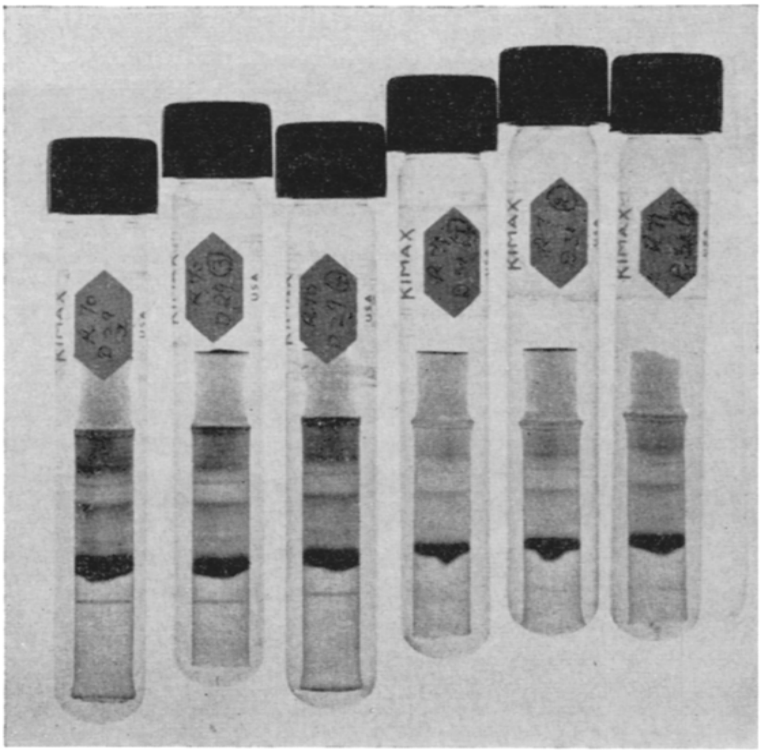

Fig. 2. Human blood serum electrophoretic patterns. D 29-1, 3, 4, D 31-1, 2, 3, human serum identical samples using incompletely polymerized sample gel, 3.9 μl, 24 min at 5 mA.

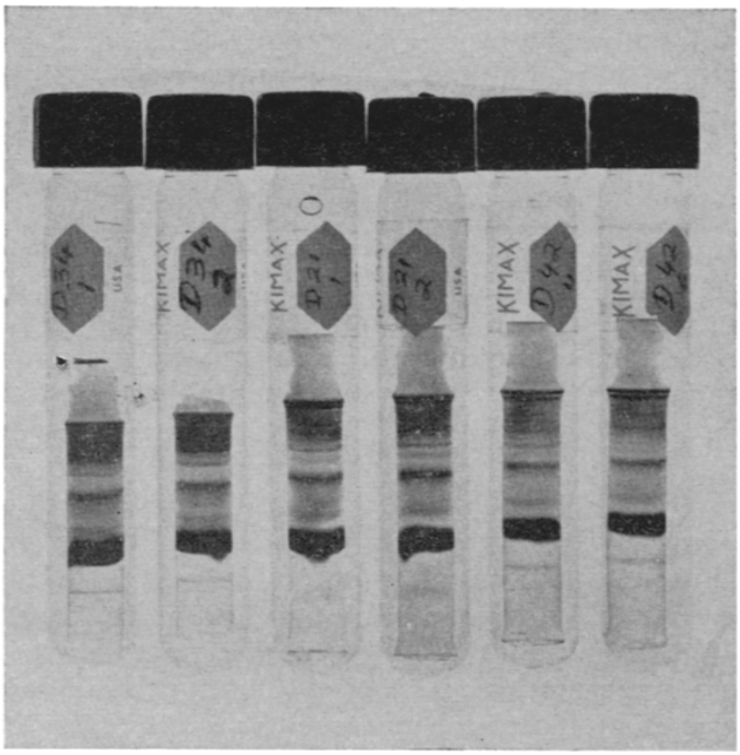

Fig. 3. Human blood serum electrophoretic patterns. D 34-1 and 2 human serum 3.5 μl using sample gel, 26 min at 5 mA. D 21-1 and 2 identical sample 3.9 μl using solution B-sucrose, 115 min at 1.25 mA. D 42-4 human serum stored at —18° for one week, 2 μl, 110 min at 1.25 mA. D 42-6 identical sample stored at 3° for one week, 2 μl, 110 min at 1.25 mA.

more it has been observed by HEIDEMAN[7] that albumin could not be recovered quantitatively in the main gel under standard disc electrophoretic conditions using sample gel. It therefore appeared to us that it would be desirable to eliminate this polymerization step by diluting the sample with solution B-sucrose and by layering it over the spacer gel. Fig. 3 D 34-1, 34-2 and D 21-1 and 21-2 provide a comparison of two procedures, the first two are a typical run using completely polymerized sample gel and the conditions described by ORNSTEIN AND DAVIS, and the latter two using solution B-sucrose solution and the present experimental conditions. It was evident that the latter patterns were better resolved and showed more definition. This may be explained as being due to partial denaturation of γ-globulins caused by the heat generated during the run D 34. D 34-1 and 34-2 may also be compared with D 42-4 and 42-6. The latter were run at 1.25 mA/tube for 110 min and at a decreased sample concentration of 2 μl per tube. While this procedure decreased the background stain it also produced increased spreading of the bands due to diffusion.

Fig. 3, D 42-4 and 6 depict differences in the serum protein pattern upon storage in a freezer (—18°) and at 3° for one week. Similar changes were observed in a subsequent run with samples of serum stored for three weeks (Fig. 4, D 51-2 and D 51-7

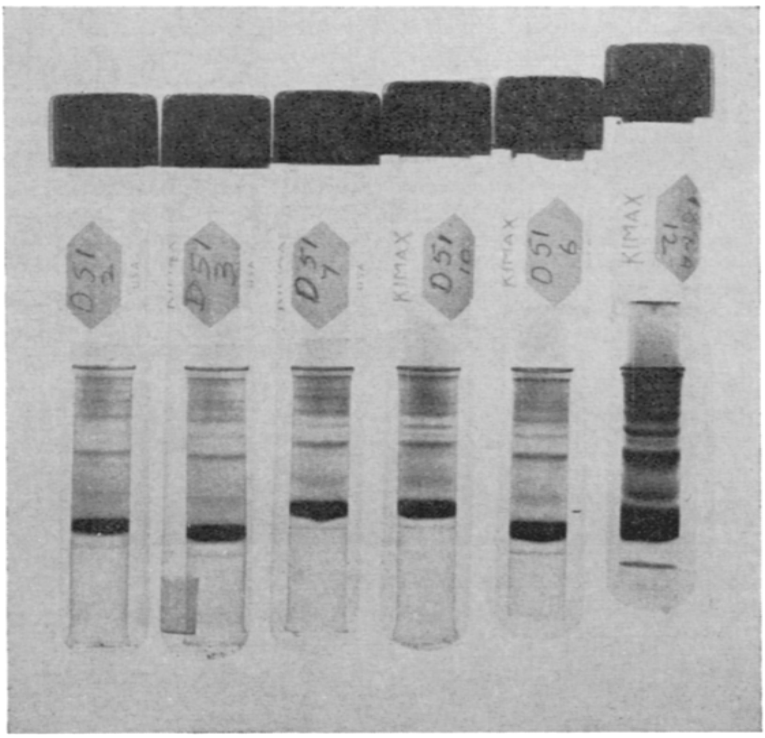

Fig. 4. Human blood serum electrophoretic patterns. D 51-2 and 7 human serum stored at —18° for 3 weeks, 1.9 μl, 55 min at 2.5 mA. D 51-3 and 10 identical sample stored at 3° for 3 weeks, 1.9 μl, 55 min at 2.5 mA. D 51-6 identical sample diluted with solution B-sucrose and stored at —18° for 3 weeks, 1.9 μl, 55 min at 2.5 mA. 189A-12 human serum sample 7 μl, 56 min at 2.5 mA.

frozen sample *vs.* D 51-3 and D 51-10, 3° sample). These were run at the lower concentration but at a higher current (2.5 mA) in order to reduce spreading. In general disappearance of some of the globulin bands was followed by appearance of new fine bands. These changes are less pronounced in the photographs but are clearly visible in

the gels. Although some of the bands in Fig. 3, D 21 are sharper by comparison because of higher concentration, the finer bands are masked due to intense background stain. This stain cannot be removed either electrophoretically or by extensive washing and may represent unresolved migrating protein components or labile protein denatured by heat, pH or small ionic strength changes. This effect was minimized by using the lowest possible serum concentration and lowest possible current (Fig. 4, D 51). Since it is difficult to store small quantities of serum as such, it was considered worthwhile to study the effect of storage of serum diluted in "solution B-sucrose" solution. D 51-6 represents a sample which was kept frozen for 3 weeks in this manner. The electrophoretic pattern was observed to be similar to that of undiluted serum frozen as such. The present method does not restrict one to low concentrations of samples as in the case of sample gel. Fig. 4 189A-12 represents a sample of human serum run at a concentration of about 7 μl.

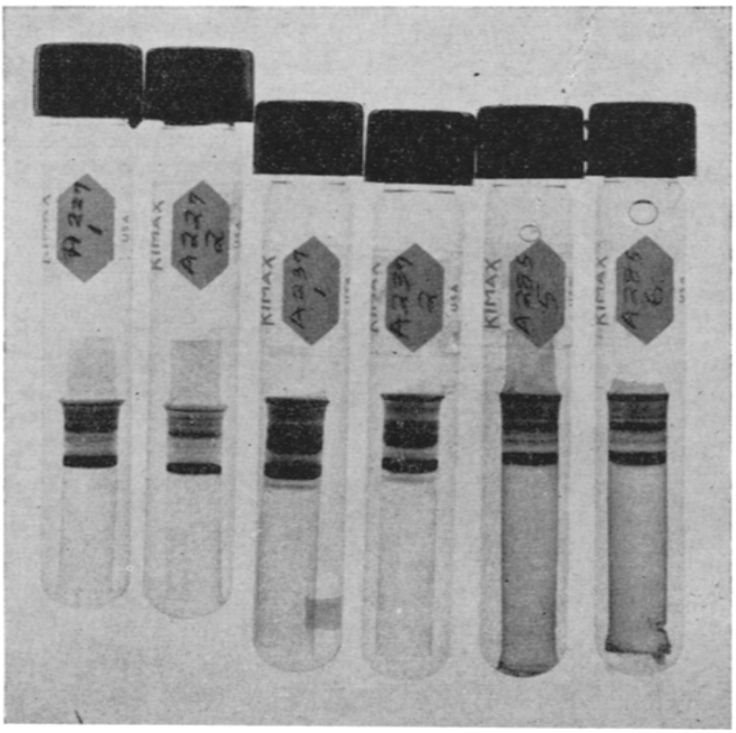

Fig. 5. Animal blood serum electrophoretic patterns. A 227-1 and 2 rat serum 2.4 μl, 30 min at 2.5 mA. A 237-1 and 2 chick serum 2.4 μl, 30 min at 2.5 mA. A 285-5 and 6 mouse serum 3 μl, 30 min at 2.5 mA.

Although quantitative aspects of the gel patterns have not been discussed here, preliminary work using Model E Microdensitometer have indicated reproducibility of the patterns under present experimental conditions. Thus with this technique only 4 μl of *whole blood* need to be drawn from either man or animal to obtain a complete electrophoretic blood serum picture. This amount may be compared with 20-50 μl of *serum* required for normal starch gel electrophoresis. The method was observed to be rapid, more convenient and simpler than the normal clotting method which not only requires greater quantities of blood but may partially hemolyse and interfere with sample gel polymerization. Finally it permits periodic examinations of the blood

serum protein pattern and may therefore find application in clinical work and in animal experimentation.

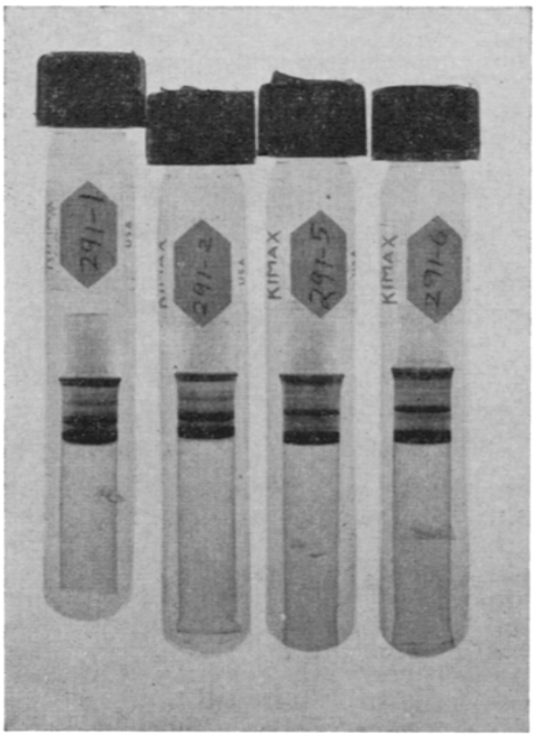

Fig. 6. Animal blood serum electrophoretic patterns. 291-1 and 2 guinea pig serum 3.6 μl, 35 min at 2.5 mA. 291-5 and 6 rabbit serum 3.6 μl, 35 min at 2.5 mA.

SUMMARY

A modified disc electrophoretic method has been developed and the serum electrophoretic patterns of chick, rat, mouse, guinea pig and rabbit have been obtained from micro quantities of blood. The modification involved use of sucrose solution rather than the large pore gel as the anti-convection medium and thus eliminated the problem of incomplete sample gel polymerization. The method described required as little as 4 μl of whole blood for a complete run, was more convenient and rapid than the usual methods and may find application in animal experimentation and in clinical work.

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