XCVII. ELECTROPHORESIS OF HUMAN BLOOD PLASMA ELECTROPHORETIC PROPERTIES OF FIBRINOGEN

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TISELIUS [1937, 1, 2] has described a new electrophoresis apparatus and its application to the analysis of the protein fractions in native horse serum. The new apparatus makes disturbance-free electrophoresis possible and components having different mobilities can be isolated. An important advantage of the apparatus is that the migrating protein boundaries are rendered directly visible by means of the Toepler "Schlieren" method.

The analysis of human blood plasma has now been undertaken, with the Tiselius apparatus. In the case of plasma one expects to find at least one more component than in serum, i.e. the fibrinogen. The statements in the literature regarding the electrochemical properties of this plasma constituent disagree widely [v. e.g. Mainzer, 1932]. Isoelectric points between pH 3.9 [Farkas & Groak, 1929] and pH 12.5 [Rossier, 1928] have been given. Kylin & Poulsen [1936] found three different fibrinogens, with isoelectric points at pH 5.4, 8.5 and about 12.4 respectively. The reasons for these discrepancies have been discussed by Kylin & Poulsen [1936] and others, and seem to lie mainly in differences with regard to the method used, the electrolyte milieu and the preparation of the fibrinogen.

The fibrinogen seems to be more sensitive towards thermal and chemical treatment than most other proteins. Even freezing, which has been found by Tiselius to produce no change in the electrophoretic properties of horse serum proteins, changes human blood plasma. After freezing there appear thick, cloudy precipitates and the normal electrophoretic diagram is not obtained. All plasmas tried behaved similarly in this respect. Human serum does not change its properties even after repeated freezing and there very seldom appear any precipitates. (It is necessary to draw the blood from fasting persons otherwise the colloidal fatty particles appearing in plasma after food intake may render the optical measurements impossible.)

For the reasons mentioned, only fresh oxalated plasma was used. The blood cells were centrifuged off immediately after the blood was drawn. For the serum experiments, the blood was left for about half an hour before centrifuging, in order that spontaneous clotting and subsequent contraction of the fibrin could take place. For mobility studies plasma and serum respectively were diluted four times with the buffer to be used and then dialysed overnight at 0° against a large volume of buffer solution. Acetate and phosphate buffers of constant ionic strength 0.1 were used.

The electrophoresis was carried out at 0° with a potential gradient of about 7 volt/cm. Rubber stoppers were fitted in the open ends of the electrode vessels

to reduce their areas as much as possible in order to counteract hydrostatic displacement, and all other conditions for disturbance-free electrophoresis were maintained. To make all boundaries visible from the beginning of the experiment, before switching on the current the plasma-buffer boundary was moved forward a short distance by means of a very slow injection of buffer solution into one of the electrode vessels. A slowly migrating boundary might otherwise remain hidden behind the cell end-plates during a whole run.

In horse serum Tiselius found, besides the albumin, three cataphoretically different globulins, called α , β and γ respectively. On the positive side there also appeared a fifth boundary, called δ , of almost zero mobility, which was observed in undiluted as well as in four times diluted horse sera. As all attempts to isolate the corresponding protein component failed, it was thought to represent some sort of equilibrium compound. The appearance of the δ boundary, however, has now been found to be due mainly to a conductivity disturbance which has often been observed in the electrophoresis of concentrated solutions [Tiselius & Svensson, 1938].

The electrophoretic diagram of human serum at pH 7.91 is seen in Fig. 1. Four different boundaries are visible, symmetrical on both sides. These boundaries correspond to albumin, globulins α , β and γ respectively. In four times diluted human serum no δ -boundary is visible, although it appears in undiluted or twice diluted serum.

The photographs in Fig. 3 were taken after migration for 44 min. at 7.9 volt/cm.

The plasma diagram obtained under the same conditions is shown in Fig. 2. The plasma and serum from which the diagrams are obtained, were taken at the same time from one person, dialysed against the same buffer and subjected to electrophoresis under as identical conditions as possible. In the plasma diagram there appears on both sides, between the β and γ boundaries, a new boundary. As the diagrams are otherwise exactly similar and as, in addition, the electrophoretic mobilities of albumin and globulins α , β and γ respectively are found to be, within the limits of the experimental errors, identical in plasma and serum, the new boundary must doubtless be due to the fibrinogen. (In order to bring out all components in the photographs, the Schlieren diaphragm has to be set in an intermediate position, hence the photographs do not show the maximum

boundary sharpness obtained with the screen in optimum position for a single boundary.) In Fig. 3 plasma and serum diagrams are placed side by side. Plasma shows the fibrinogen behind the β boundary. In this figure globulin γ is not visible, being hidden behind the end plates of the cell. In all photographs, the migration direction is downwards.

The electrophoretic mobilities of the plasma and serum components as a function of pH are plotted in Fig. 4. Each point is the mean of several experiments on serum and plasma. Only sera and plasma from apparently healthy persons were used. The mobility data are also given in Table I.

Fig. 4.

The isoelectric points given in Table I are very approximate only. In the mixtures of proteins, the isoelectric points determined cataphoretically differ somewhat from those found with the isolated protein, owing to mutual interaction or other factors.

Table I

Electrophoretic mobilities, in $\text{cm}^2 \text{V}^{-1} \text{sec}^{-1} \times 10^5$, of the protein components in normal human plasma and serum at different pH . Ionic strength 0.1. Temperature $\pm 0.0^\circ$.

Buffer	pH	Albumin	Globulin α	Globulin β	Fibrinogen	Globulin γ
Acetate	5.2	-2.0	—	-0.1	—	+1.5
Phosphate	5.6	-3.2	-2.4	-0.9	—	+0.5
"	6.1	-3.9	-3.0	-1.7	-0.9	+0.2
"	6.9	-5.5	-4.4	-3.0	-1.5	-0.1
"	8.0	-7.7	-6.1	-4.2	-2.5	-0.4
Isoelectric points pH	—	—	(4.8)	5.2	5.4	6.4

Under the experimental conditions employed, no separation of globulin α from the albumin, below pH 5.5, could be detected with the optical arrangement used. In general, globulin α migrates more closely with albumin in human plasma than it does in horse serum; furthermore, its concentration in human serum is very low, being roughly about 0.25% in concentrated serum (in horse serum about 0.95%). The globulin β boundary is always associated with a yellow-brown colour and a slight opalescence, which shows up against the yellow colour of the albumin (due to bilirubin [Bennhold, 1932; Pedersen & Waldenström, 1937]). In plasma the globulin β boundary always tends to spread out towards the fibrinogen boundary. Below pH 5.7 the fibrinogen boundary does not separate from the β boundary, which appears broad and rather diffuse.

The globulin γ is always colourless. The slow migration of this component at a more alkaline reaction, as compared with horse serum, may possibly be due to a slight δ -effect. On account of the low concentration of globulin α and fibrinogen in plasma, no attempt was made to isolate all components with the rather small cells at present available. It is hoped to make use of a larger cell in the near future. Therefore albumin only was isolated, the mobility curve of which is given in Fig. 5.¹ The isoelectric point is at pH 4.64, which is the same as found by Tiselius for horse serum albumin.

Fig. 5.

The isoelectric point of the fibrinogen in the protein mixture of plasma lies in the region of pH 5.2–5.6, its exact value cannot be obtained from these experiments for the reasons stated above. The approximate value arrived at is in agreement with that found by Nordbö [1927], who used the potentiometric titration method. As Kylin & Poulsen claim to have found by means of electrophoresis two fibrinogen fractions with isoelectric points more alkaline than pH 8, a special experiment was carried out with concentrated plasma to test this, as there is the possibility that a component present only in a very low concentration in native plasma may escape detection in four times diluted plasma. (With the experimental arrangement employed the lowest concentration in which a protein can be detected optically is about 0.03–0.05 %.) Undiluted plasma was subjected to prolonged electrophoresis (5 hr. at 4 volt/cm.). At pH 7.5 no cathodic migration was observed. As a further test, the solution behind the slowest boundary was isolated and tested with salicylsulphonic acid. No trace of protein could be found. The claim of Kylin & Poulsen must be considered in the light of the fact that in their experiments the conditions for disturbance-free electrophoresis were not maintained (undiluted plasma, unbuffered solutions, long runs at a relatively high temperature etc.).

An approximately quantitative estimation of fibrinogen in plasma gives a value in good agreement with those ordinarily found under normal conditions, i.e. 0.2–0.4 %.

SUMMARY

Human blood plasma and serum have been subjected to electrophoretic analysis by means of the new apparatus of Tiselius. Serum was found to contain, in addition to the albumin, three cataphoretically different globulins,

¹ An analysis of this albumin in the ultracentrifuge, kindly performed by Dr K. Pedersen of the Institute of Physical Chemistry, Upsala, also showed it to be free from globulin.

corresponding to those found by Tiselius in horse serum and called α , β and γ respectively. Plasma, besides the components mentioned, contains one more component, the fibrinogen. This was found to migrate more slowly than β globulin but faster than γ globulin and to be an electrophoretically well-defined protein having its isoelectric point at about pH 5.4. The electrophoretic mobilities of the plasma and serum proteins over the pH range 5–8 are given, together with the approximate isoelectric points. The isoelectric point of electrophoretically isolated serum albumin was found to be at pH 4.64. Some earlier results by other investigators are discussed.

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